University of Alberta

FATIGUE AND UNDER-PERFORMANCE IN ELITE COMPETITIVE SWIMMERS: TOOLS FOR MONITORING TRAINING

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

Faculty of Physical Education and Recreation

Edmonton, Alberta

Spring, 2004

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ISBN: 0-612-96561-9
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ABSTRACT

This study investigated fatigue, under-performance, and tools for monitoring training in 6 male and 8 female elite competitive swimmers (aged 16-23 years) over a 10-week period. Tools investigated included hematological, immunological, hormonal, cardiovascular and performance measures. Analysis of the group results was useful in determining changes that took place across a training macrocycle. Analysis of the results for the 3 female responders (R) (i.e., optimally trained) compared to the 3 female non-responders (NR) (i.e., poorly trained) was useful for delineating normal from abnormal training responses. The results for the NR revealed that these athletes were experiencing a very high level of fatigue, especially during the taper period. Final analyses involved a case study approach to examine the outlying data of one athlete based on her under-performance at the major competition. The results from this subject provided support for the use of a multidisciplinary approach to assess fatigue and overtraining.

ACKNOWLEDGEMENTS

I would like to thank the following people for helping me to succeed:

My Mom – for her help in so many ways; for the numerous revisions; for all the late nights working with me; for pushing me to finish; for worrying about my project as much as I did; for her love and support; and for being a wonderful friend.

My Dad – for all his support and understanding; for never letting me give up on my goals and dreams; for helping me through the hard times; and most importantly for all his love.

Dr. Dru Marshall – for being a wonderful advisor and friend; for all her help with testing; for her many revisions and suggestions; for being patient and understanding of my situation; for teaching me to be confident in my work; and for helping me to grow as a person.

Dr. Steve Norris – for all the hours spent instructing me; for the many trips to Edmonton; for helping with the testing sessions; for providing me with great suggestions, and for helping me develop a very interesting study.

Dr. Vicki Harber – for being a wonderful instructor who initially sparked my interest in exercise physiology; and for all her help with the cortisol analysis.

Dr. Catherine Field – for her wonderful suggestions and revisions; for providing me with excellent resources; and for initiating my interest in immunology and nutrition.

Dr. Gordon Bell – for teaching me the lab techniques that I needed; for being a terrific instructor; and for always being supportive and encouraging.

Susan Goruk – for her hours of help in the lab; for teaching me numerous assays; and for keeping me company through it all.

Kelvin Lien – for all his time spent teaching me to use high performance liquid chromatography.

Deb Harding – for collecting and performing the dietary analyses; for sharing all the information that she obtained; for teaching me about nutrition; for providing me with the papers that she wrote; for helping with testing sessions; and for presenting the dietary information to the swimmers.

Marc Tremblay – for being a wonderful coach and boss over the years; for encouraging me to pursue my Masters; for teaching me how to be dedicated; for helping me with testing; and for being passionate about my results.

John Vadeika – for all his help with the swim testing; for being very accommodating with testing days; and for encouraging the swimmers to participate in my study.

Loralie Norton – for collecting all of the blood samples for me and for relieving a lot of my stress.

Dr. Janet Yoneda – for generously requesting and interpreting many of the blood results.

Huiqin Hu – for teaching me what statistical analyses to use; for helping me to run the program; and for making sure that I interpreted my data correctly.

Carl, Jackie & Andrew – for their assistance with timing swimming test sets; for coming to early morning test sessions just to help out; for their interest in the results; and for their love for swimming.

Lisa Workman – for her help with testing sessions.

My Sister – for her many words of wisdom; for her support and encouragement; and for being a terrific friend.

Trevor – for being a wonderful friend and confidant.

And lastly...to the 14 swimmers who willingly gave their time and shared their personal information. I wish them all continued success in their swimming careers.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic Hormone
ANOVA	Analysis of Variance
BCAA	Branched Chain Amino Acids
BMI	Body Mass Index
CBC	Complete Blood Count
CD	Clusters of Differentiation
CNS	Central Nervous System
CRH	Corticotropin-Releasing Hormone
CV	Coefficient of Variation
ECG	Electrocardiogram
GI	Gastrointestinal
GU	Genitourinary
Hb	Hemoglobin
Hct	Hematocrit
HF	High Frequency
H-P-A	Hypothalamic-Pituitary-Adrenal
HPLC	High Performance Liquid Chromotography
HRV	Heart Rate Variability
IF	Immunofluorescence
IgA	Immunoglobin A
LF	Low Frequency
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
NK	Natural Killer
NR	Non-Responders
PCV	Packed Cell Volume
PHSC	Plutipotential Hemopoietic Stem Cells
R	Responders
RBC	Red Blood Cell Count
RIA	Radioimmunoassay
SD	Standard Deviation
SE	Standard Error
S-IgA	Salivary Immunoglobin A
TCA	Tricarboxylic Acid
TIBC	Total Iron Binding Capacity
UASC	University of Alberta Swim Center
URTI	Upper Respiratory Tract Infection
VLF	Very Low Frequency
VO_2	Exercise Oxygen Consumption
VO_2max	Maximal Exercise Oxygen Uptake
WBC	White Blood Cells

CHAPTER ONE: INTRODUCTION

OVERVIEW:

Athletes are continually trying to enhance their performance by pushing their capacity to its upper limits (Kuipers, 1998). Endurance athletes especially try to endure high volumes of strenuous training in order to elicit positive physiological adaptations. Unfortunately, there is no clear boundary between positive training, which results in improved performance, and overtraining, which results in performance decrements (Kreider et al, 1998).

In brief, overtraining is a serious problem affecting between 7% - 20% of high performance athletes (Mackinnon, 2000). Overtraining is the term which indicates that an individual has been stressed by training or extraneous factors to the extent that he or she is unable to perform at an optimal level following a prolonged period of rest (i.e. greater than 2 weeks) (Fry et al, 1991a). Generally, overtraining is characterized by excessive fatigue, persistent muscle soreness, mood disturbances, and a feeling of being "burnt out" (Uusitalo, 2001). This is a serious problem as even a small decrement in performance can be catastrophic to an athlete who has invested years working towards a major competition. Poor performances at critical times may influence selection for teams, lead to loss of government funding, or cause the athlete to prematurely retire from the sport (Mackinnon).

Overtraining has been investigated in a variety of sports, ranging from marathon running to resistance training. The prevalence of overtraining is higher in endurance sports requiring both high volume and intensity training, such as swimming, running, and cycling (Mackinnon, 2000). The strongest determinant of overtraining appears to be a progressive

increase in training intensity, as well as a significant increase in training volume, without sufficient recovery time (Uusitalo, 2001).

Several researchers have examined markers of overtraining or decreased performance capacity. Some of the parameters which have been investigated include: heart rate, heart rate variability, and blood pressure; exercise oxygen consumption (VO_2); sports specific performance measures; and blood levels of hemoglobin, red and white cells, lactate, iron, ferritin, glucose, urea, various enzymes, amino acids and various hormones (Hooper & Mackinnon, 1995). As is clear from the significant number of parameters investigated, no single marker has been found to be universally useful for the diagnosis of overtraining. Therefore, a multidisciplinary approach, which utilizes a combination of physiological, biochemical, immunological and hematological parameters is needed to help clarify overtraining (Rowbottom et al, 1996).

While it is important to identify markers of overtraining, it is equally important to develop scientific testing programs that can be incorporated into an athlete's training regime for early detection of overtraining (Kreider et al, 1998). Prevention of overtraining can be accomplished through careful control of the training program. This necessitates the identification and regular use of appropriate tools for monitoring training in order to prevent overtraining (Fry et al, 1991a).

Proper monitoring of training provides useful feedback to coaches and athletes regarding actual training. Monitoring of training helps to determine if a training design is adequate for an individual athlete at a specific stage of training (Viru & Viru, 2001). As well, due to the fine line between effective training and overtraining, the regular monitoring of training supplies coaches and athletes with information regarding the adaptability of the

body, including the diagnostics of early manifestations of overtraining (Viru & Viru). Therefore, coaches and athletes need to monitor training on a regular basis. Since many of the proposed tools for monitoring training are relatively expensive (e.g., hormonal and hematological measurements), there is a need to find convenient, inexpensive, valid and reliable markers that provide early detection of overtraining. Using these markers, coaches could then adjust training loads to avoid overtraining.

PURPOSE:

The purpose of this study was to investigate an intact squad of elite competitive swimmers using various tools for monitoring training and assessing fatigue during a 10-week macrocycle. In particular, the tools that were investigated included hematological (red blood cell and iron indices), immunological (white blood cell and differential, salivary immunoglobin A, and glutamine/glutamate), hormonal (cortisol), cardiovascular (dynamic postural heart rate test and spectral heart rate analysis) and performance measures (test sets and time trials). This study analyzed these aforementioned variables in order to discern overall trends across the different phases of training (i.e. baseline, build, crash and taper). This study also attempted to identify and distinguish between changes associated with overtraining and changes associated with optimal performance. In other words, this study strived to delineate the normal versus the abnormal training responses of the aforementioned variables by comparing optimally trained to poorly trained athletes. Further, this study attempted to identify the most sensitive markers of fatigue, and the most probable markers of overtraining.

HYPOTHESES:

It was hypothesized that a significant increase in training volume and intensity during a crash (i.e. overload) training period would cause changes in a variety of measures including:

Hematological Measures:

- decreased red blood cell, hemoglobin and hematocrit levels
- decreased iron and ferritin

Immunological Measures:

- decreased number of leukocytes,
- decreased proportion of T-Cells (CD4+/CD8+ ratio)
- decreased proportion of natural killer cells;
- decreased salivary IgA,
- decreased resting plasma glutamine concentrations,
- increased resting plasma glutamate levels;
- decreased ratio of glutamine to glutamate;

Hormonal Measure:

- increased resting plasma cortisol levels;

Cardiovascular Measures:

- a change in autonomic nervous system activity showing increased sympathetic activity (as measured by spectral heart rate analysis and/or the dynamic postural heart rate test)

Performance Measures:

- decreased performance in the test sets (4 x 50m and 5 x 200m) and time trials (100m maximal effort swim)

It was also hypothesized that for optimally trained athletes, a reduction in training volume/intensity along with sufficient recovery as provided in a taper, would reverse the effects of overload training and prevent the development of overreaching or overtraining.

However, in overtrained athletes, it was believed that a taper period would not provide sufficient rest to allow for a reversal of the effects. As a result, it was hypothesized that overtrained athletes would perform poorly at the major competition at the end of the 10-week study.

SIGNIFICANCE OF THE STUDY:

This was a longitudinal study (10 weeks) that used a multidisciplinary approach to examine competitive swimmers in their natural training environment. According to Mackinnon (2000), longitudinal studies are advantageous as athletes may be assessed at various times throughout a competitive season. In this study, the subjects were assessed 5 times during the 10-weeks. Specifically, two testing sessions occurred prior to the start of training in order to establish baseline values. The subsequent testing occurred during a build period, a crash period, and finally a taper period. The significance of this type of design was that the physiological responses could be compared within individual athletes between testing periods. As well, this design allowed for comparison between those athletes considered optimally trained and those athletes showing signs of overtraining.

Further, this study was significant as it attempted to control for some of the methodological errors occasionally made in overtraining studies. Overtraining research has been very controversial to date (Uusitalo, 2001), as studies have produced results that are inconsistent, making definite conclusions difficult (Hopper & Mackinnon, 1995). According to

Tremblay & Chu (1994), research discrepancies may be explained by methodological inconsistencies. In order to minimize interference by confounding variables, researchers must control for factors such as time of day of testing, diet, testing environment, training history, training volume and intensity, time interval to previous exercise, and use of caffeine, tobacco or other substances (Tremblay & Chu; Uusitalo). Therefore, this study attempted to standardize testing methodology based on the recommendations in the literature.

Also of critical importance was the fact that this study used a combination of expensive, invasive measures and non-invasive, more affordable measures that coaches could use. This was imperative, as the study of overtraining needs to incorporate practical measures that coaches can use on a regular basis to monitor training. It is hoped that this study will provide important and useful information to the swimming community regarding the monitoring of training, with the ultimate goal of preventing performance decrements.

SCOPE OF THE STUDY:

This study investigated a unique variety of parameters, which to date have not been examined in this particular combination. The independent variables included the volume and intensity of swim training for a 10-week period. The dependent variables included hematological (red blood cell and iron indices), immunological (white blood cell and differential, salivary immunoglobin A, and glutamine/glutamate), hormonal (cortisol), cardiovascular (dynamic postural heart rate test and spectral heart rate analysis) and performance measures (test sets, time trials, and the major competition). These particular

tools were selected either because they appeared to be the most common in the literature, or else they were promising new parameters which needed further investigation.

LIMITATIONS:

Limitations to this study revolved around the fact that the researchers could not manipulate the training. The coaches determined the volume and intensity of the practices, and as a result each swimmer did varying amounts of training. This is due to the fact that it is unethical to purposely overtrain an athlete. Other limitations to this study included the fact that it was difficult to control for confounding variables, such as illness, diet, travel, sleep, and competition stress. Another disadvantage was that this study was limited to competitive swimmers. As such, it is difficult to determine how well the information collected will be representative of other sports.

DEFINITIONS:

Standardized terminology should be encouraged in overtraining literature. The terms utilized in this study (which have been adapted from Fry et al, 1991) are as follows:

- Overload training - stressing an individual to produce a stimulus for adaptation and supercompensation. This is necessary for improved performance.
- Overreaching - the induction of short term overtraining, which can be reversed by a longer than normal regeneration period.
- Overtraining - further exposure to training and extraneous stressors to the extent that the individual is unable to perform at an optimum level following a period of prolonged rest (i.e. greater than 2 weeks).

- Overtraining syndrome – a state of chronically depressed performance accompanied by one or more serious physiological/ psychological symptoms.

Other pertinent terms that are used throughout this study include:

- Fatigue – a physiological state developed as a result of intense or prolonged activity and manifested by a decrease in work capacity, a feeling of tiredness and discordance of functions (Viru & Viru, 2001).
- Under-performance - performance by an athlete that is below the athlete's potential and that is at a level below what has previously been achieved.
- Optimally trained - a state of training that enables an athlete to perform at an optimal level during major competitions as a result of an effective training program.
- Baseline – the time period prior to the start of the training season (e.g. off season)
- Build – a training phase distinguished by a uniform increase in training loads with considerable volume, but below the ultimate level of intensity in most training sessions (Fry et al, 1992).
- Crash – a training phase distinguished by considerably high volume and intense training sessions (Fry et al, 1992), and frequently referred to as overload training.
- Taper – a period where training volume is incrementally reduced, often preceding a competition (Houmard & Anderson Johns, 1994).

CHAPTER TWO: LITERATURE REVIEW

OVERVIEW:

The purpose of this chapter is to carefully examine the literature relating to fatigue, under-performance and overtraining. Specifically, this chapter has been divided into 11 parts. The first part examines the principles of training that are necessary for improved performance, and leads into an explanation of the training-overtraining continuum. The second part examines the concept of overtraining, including the different types of overtraining, its prevalence in elite sport, as well as the factors that increase susceptibility to overtraining. The third through eighth parts examine a variety of tools for monitoring training stress and fatigue in order to prevent overtraining. Specifically, these sections critically examine the specific variables that were tested in this study, which include a combination of hematological, immunological, hormonal, cardiovascular and performance measures. Each of these areas is discussed in a separate section in the order listed above. Finally, the last three sections deal with methodological considerations pertinent to overtraining research, directions for future research and a summary of the literature review chapter.

PART I - TRAINING

TRAINING PRINCIPLES:

The primary goal of coaches and athletes is to optimize training in order to enhance performance. When the intensity, duration and volume of training is appropriate, positive physiological adaptations occur, and performance typically improves (Armstrong & Van Heest, 2002). In order to accomplish this, coaches need to design and implement training

programs that are appropriate to the needs and level of each individual athlete. There is no training blueprint that is ideal for all athletes or sports. There are, however, basic training principles which provide a framework on which to base training recommendations (Thompson, 2001). Four basic training principles which will help to optimize performance include overload training, specificity, periodization, and tapering.

A) OVERLOAD TRAINING:

Improvements in an athlete's performance are achieved through training, which induces physical, physiological, and psychological stress. Applying a series of stimuli will displace the homeostasis of the athlete's functional systems, providing a stimulus for adaptation (Fry et al, 1991a). This is achieved through overload training. *Overload training* refers to a planned, systematic, and progressive increase in training stimuli (Armstrong & Van Heest, 2002). These training stressors disrupt the stability of the athlete's internal environment, producing physiological adaptations called training effects (Thompson, 2001).

Following overload training, there is a *restoration* period in which the athlete strives to re-establish homeostasis. During this time, the cells, tissues and organs that were disturbed during the overload training are restored (Thompson, 2001). The length of this period depends predominantly on the degree to which the stress was applied (Fry et al, 1992), but also depends on the athlete's fitness level and nutritional status; the intensity, duration and frequency of the exercise; and on genetic factors (Thompson).

Subsequent to restoration, an *adaptation* phase occurs. During this phase, the various cells, tissues and organs that were disturbed undergo changes in their structure and function. This permits the athlete to exercise at a heightened work capacity, above his or

her previous capability (Fry et al, 1991a). According to Thompson (2001), some of the physiological adaptations that occur, depending on the stimulus, include: increased blood volume, increased heart size, maximal stroke volume, and maximal cardiac output; as well as increases in size, strength, enzymatic activity, capillary number and fuel capacity of the exercised muscle.

This sequence of overload training, followed by restoration and adaptation is referred to as the *supercompensation* cycle. Each cycle gives rise to an improved fitness level, which hopefully will lead to improved performance (Thompson, 2001).

B) *SPECIFICITY:*

The principle of specificity is one of the most important training principles. According to this principle, the physiological adaptations that occur with training are highly specific to the type of activity performed, the systems stressed, the motor units recruited and their recruitment pattern, and the volume and intensity of exercise. For instance, swim training results in several muscular adaptations that are not necessarily going to make someone a better and more efficient runner. Therefore, training programs need to focus on the development of the fitness attributes that are critical for optimal performance in that particular sport (Thompson, 2001). In swimming, the intensity and duration of the event determines the relative contribution of the 3 energy systems (the alactic, lactic, and aerobic systems) (Pyne et al, 2000b). As a result, training must be designed differently for sprint swimmers and distance swimmers.

C) PERIODIZATION OF TRAINING:

Periodization of training is the most reliable way of eliciting positive performance while minimizing the likelihood of decrements (Armstrong & Van Heest, 2002). Typical training periodization divides the year into phases of varying duration. Fry et al (1991a) suggested that a training year be divided into macrocycles, mezocycles, microcycles, and training units. Macrocycles divide the year into three major periods: preparation, competition and transition. During these periods there is generally a gradual change from predominantly distance and strength work, to more intense and sport-specific workouts later in the season. These macrocycles are further divided into mezocycles, which are usually 4 week training blocks. Mezocycles generally consist of four microcycles, which are one-week training blocks. The first two microcycles are often developmental (also known as build periods), the third is a crash cycle (very hard training), and the fourth is an unloading cycle. This means that there are three weeks of increasing volume and intensity of training, followed by a fourth week of recovery (Kreider et al, 1998). Lastly, training programs can be narrowed down into individual training sessions (Fry et al, 1992).

D) TAPERING:

The macro-, mezo-, and microcycles of periodized training allow for regeneration to occur following training overload. The regeneration from the negative aspects of training is an important focus of *tapering*, which is a period of reduced training before a major competition (Hooper et al, 1999). According to a review article by Houmard & Anderson Johns (1994), a successful taper includes a graded reduction in training volume of 60% - 90%, and an increase in daily high-intensity work for a 7 to 21 day period. As well, training

frequency should be reduced by approximately 20% -50%. Following these general guidelines during the taper will typically enhance the possibility of optimal performance.

THE TRAINING/OVERTRAINING CONTINUUM:

The preceding review has suggested principles which are the foundation of successful training. However, these same basic training principles also have the potential to cause performance decrements in athletes. Despite elaborate training programs, which correctly utilize the principles of overload, periodization, and tapering, even the most experienced coaches admit that it is very difficult to predict which athletes will experience lasting performance decrements (Armstrong & Van Heest, 2002). It has been suggested that there is a training /overtraining continuum, in which there is a subtle border between training which elicits optimal performance, and overtraining which leads to performance decrements (Fry et al, 1991; Armstrong & Van Heest). This continuum is comprised of different levels of training, ranging from undertraining to overtraining (Figure 2.1) (Armstrong & Van Heest). *Undertraining* refers to the rest time between seasons or to a period of active rest. *Overload* training, which has been described in the preceding paragraphs, is a planned increase in training stimuli, while *overreaching* refers to a brief period of overload, during which there is insufficient recovery time. Overreaching usually results in performance decrements for a few days or weeks. If the fatigue associated with this period is reversed within this relatively short period of time, then the athlete will typically perform at a level higher than previously achieved (Armstrong & Van Heest). The final stage in the continuum is *overtraining*, which is characterized by diminished sports-specific performance, accelerated fatigability and subjective symptoms of stress, despite a

a prolonged period of rest (greater than 2 weeks) (Urhausen et al, 1995). As can be observed by Figure 2.1, there is a fine line between optimal training and overtraining (see dotted line at right). This suggests that athletes at their peak are also on the verge of overtraining (Armstrong & Van Heest). This concept of overtraining will be discussed further in the next section.

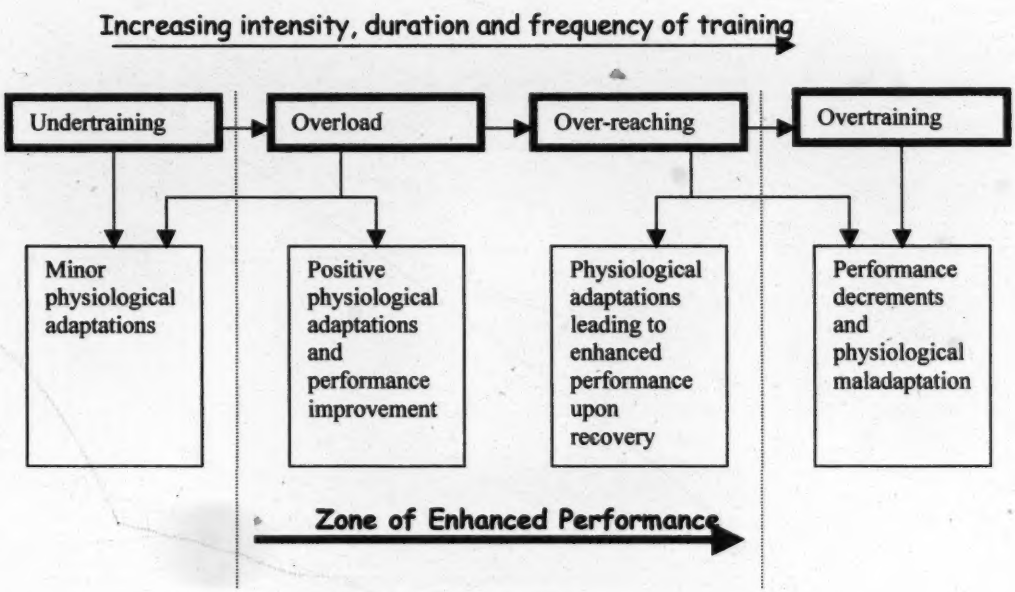

Figure 2.1 The Continuum of Training States. Adapted from Armstrong & Van Heest (2002).

PART II - OVERTRAINING

PREVALENCE OF OVERTRAINING:

The prevalence of overtraining is difficult to determine precisely as it requires numerous studies of athletes from diverse sports. It has been suggested that between 7% and 20% of all athletes may demonstrate symptoms of the overtraining syndrome (Mackinnon, 2000). The prevalence also appears to vary between sports. Some studies

postulate that overtraining is higher in endurance sports requiring high volume and intensity training, such as swimming, rowing and distance running. As well, sports which are body mass supported (such as swimming) are more prone to causing overtraining, as opposed to weight bearing activities in which musculoskeletal injuries are more likely to limit training volume (Mackinnon).

FACTORS THAT INCREASE SUSCEPTABILITY TO OVERTRAINING:

There are certain factors that seem to make an individual more prone to overtraining. The strongest determinant appears to be a progressive increase in training intensity, as well as a significant increase in training volume, without sufficient recovery time. Some of the other factors include environmental conditions, sleep, food intake, general health, and the personality type of the individual (Uusitalo, 2001).

A) VOLUME VS. INTENSITY:

There is presently some controversy as to whether overtraining is induced more through increased intensity, increased volume, or a combination of both. Lehmann et al (1992), examined middle-distance and long-distance runners in a two-phase experimental training study, in which there was a significant increase in training intensity in one phase (9 runners, mean age 34), and a significant increase in training volume in the other phase (8 runners, mean age 33). Seven of the runners participated in both phases. In the study an increase in training volume from 85.9 Km/week to 176.6 Km/week over 3 weeks caused a considerable decrease in maximal performance, with performance incompetence for months. It was concluded that these results were probably due to overtraining. In the same study,

an increase in training intensity (e.g. the high speed and interval runs increased from 9 Km/week to 22.7 Km/week over 3 weeks) failed to cause an overtraining syndrome. Instead, the increase in intensity resulted in an increase in the subjects' endurance and a general improvement in their performance. These results are dissimilar to the results found by Urhausen et al (1998), who studied 17 male endurance athletes (cyclists and triathletes, mean age 23.4 years) 5 times over a period of 19 months. Overtraining was induced at one point during the study by a distinct increase in training intensity, without any significant increase in volume. In particular, the exercise sessions were performed at an intensity above the individual anaerobic threshold, which led to a pronounced increase in the metabolic and physiological demands (Urhausen et al, 1998). Therefore, at this time the exact contribution of training volume vs. training intensity to the development of the overtraining syndrome is unknown (Kreider et al, 1998). In future studies, it is imperative that the authors provide precise information about the intensity, volume and frequency of exercise that causes overtraining (Kreider et al).

TYPES OF OVERTRAINING STATES:

Two types of overtraining states have been identified: sympathetic and parasympathetic (Uusitalo, 2001). In sympathetic overtraining there is a predominance of sympathetic activity, which includes symptoms such as increased resting heart rate and blood pressure; decreased appetite; sleep disturbances; emotional instability; and decreased performance (Fry et al, 1991a). Parasympathetic overtraining is characterized by a predominance of parasympathetic activity (Hopper & Mackinnon, 1995). Symptoms for this type of overtraining include: low resting heart rate and blood pressure; fatigue; suppressed

heart rate, glucose, and lactate during exercise; altered hypothalamic/pituitary, adrenal/gonadal function; and decreased performance (Kreider et al, 1998). A mixture of the two forms can exist, because the sympathetic type represents an early transitory stage that shifts to the parasympathetic type after an individually varied amount of time (Urhausen et al, 1995). Some researchers have suggested that sympathetic overtraining affects mainly speed and power athletes, whereas parasympathetic overtraining affects mostly endurance athletes (Hopper & Mackinnon). The existence of both types of overtraining indicates the need to be aware of both the down-regulation and up-regulation of neuroendocrine homeostasis when attempting to diagnose overtraining in athletes (Fry et al).

PHYSIOLOGICAL DISTURBANCES CAUSED BY OVERTRAINING:

In addition to the symptoms that have been listed for the two types of overtraining, there are a number of other possible physiological disturbances. See Appendix A for a list of the major symptoms of overtraining, as set out by Fry et al (1991a). This list illustrates the complexity of the overtraining syndrome (Fry et al).

PREVENTION OF OVERTRAINING:

It is important that athletes and coaches are aware of the signs and symptoms of overtraining in order that they can make alterations to training as soon as possible. However, it is even more important for coaches and athletes to try to prevent overtraining, rather than react to its occurrence. Prevention of overtraining can be accomplished through careful monitoring of training stress and fatigue during training. This necessitates

the regular use of appropriate tools for monitoring training in order to prevent overtraining (Fry et al, 1991a).

PART III - TOOLS FOR MONITORING TRAINING STRESS AND FATIGUE IN ORDER TO PREVENT OVERTRAINING

Numerous physiological tools have been suggested as markers of overtraining or decreased performance capacity. Some of the parameters which have been investigated include: blood levels of red and white cells, hemoglobin, hematocrit, iron, ferritin, glucose, urea, lactate, creatine kinase, and glutamine; various hormones; heart rate, heart rate variability, and blood pressure; exercise oxygen consumption (VO_2); and sports specific performance measures (Hooper & Mackinnon, 1995). Unfortunately, laboratory tests are very time consuming and expensive. Therefore, testing needs to be selective and designed to produce the maximal amount of information (Fry et al, 1991a). For the purpose of this study, a multidisciplinary approach was used. The following sections examine the specific variables that were tested in this study, including a combination of hematological, immunological, and hormonal measures, as well as heart rate variability, and performance measures. These areas are discussed in separate sections in the order listed above.

HEMATOLOGICAL MEASURES

Sports hematology has made numerous advances in the past 20 years and has emerged as a subspecialty in sports medicine (Shaskey & Green, 2000). As such, coaches and sports scientists have begun to examine blood parameters as a means of monitoring training in elite athletes. Studies have demonstrated that adaptation to physical activity is

related to changes in blood cell counts, blood plasma volumes and in the specific distribution of various cell types (Viru & Viru, 2001). Specifically, studies of the red blood cells (erythrocytes) provide information on the oxygen-transport function of the blood, whereas studies of the white blood cells (leukocytes) are aimed at understanding immune activities (Viru & Viru). The following section reviews the red blood cell system and its associated indices including hemoglobin, hematocrit, mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC). In particular, the structure, function, and the effects of exercise on the aforementioned variables are examined. As well, the roles of iron and its associated variables including ferritin and total iron binding capacity are investigated. A review of the white blood cells is done when examining the immunological indices associated with fatigue in elite athletes.

A) RED BLOOD CELL COUNT AND RED CELL INDICES:

1. Production, Structure and Function of Red Blood Cells (Erythrocytes):

Blood is composed of three formed elements: red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes). Of these three elements, red cells are the most abundant, out-numbering white cells by approximately 1000:1 and platelets by around 100:1 (Higgins, 2000). The production of these formed elements is through a process known as hemopoiesis, which takes place mainly within the bone marrow (Viru & Viru, 2001). During this process, pluripotential hemopoietic stem cells (PHSC) divide and differentiate through several stages to become red blood cells, white blood cells or platelets (Higgins).

The red cells that are created have a lifespan of around 120 days, and therefore need to be constantly replenished (Higgins, 2000). On average, approximately 2.3 million red cells are produced every second throughout an individual's life (Higgins). This seems like an immense number of cells being produced, however, the average concentration of erythrocytes in the blood is 5.2×10^6 cells per μl of blood for males, and 4.7×10^6 cells per μl of blood for females (Viru & Viru, 2001).

The structure of erythrocytes is well suited to the primary function of transporting oxygen and carbon dioxide within the bloodstream (Martini & Bartholomew, 1997). The biconcave disc shape of erythrocytes allows the largest surface area for a given volume, optimizing the area for oxygen and carbon dioxide gas exchange (Higgins, 2000). Central to this function is hemoglobin, the protein contained within erythrocytes (Higgins).

2. Structure and Function of Hemoglobin:

Hemoglobin (Hb) is the oxygen-carrying pigment present in red cells, which gives blood its red color (Higgins, 2000). The Hb molecule is comprised of four protein subunits, which each contain a single molecule of a pigment called heme. At the center of each heme is an iron ion (FE^{2+}), which forms a weak, ionic link with an oxygen molecule (O_2) (Martini & Bartholomew, 1997). The iron-oxygen interaction can easily be separated, and this is how oxygen is transported from the lungs to tissue (Higgins). The transport of carbon dioxide in the reverse direction is slightly more complicated. Carbon dioxide is soluble in plasma and therefore much of the CO_2 produced is transported in the plasma. The remaining CO_2 is then transported in erythrocytes by binding to Hb (Higgins).

3. Interpretation of Red Blood Cell and RBC Indices Test Results:

There are a number of red blood cell tests that assess the number, shape and maturity of circulating erythrocytes (Martini & Bartholomew, 1997). These tests are useful in providing information about the general health of an individual, or in detecting health abnormalities (Higgins, 2000). Specifically, the tests that focus on the erythrocytes include red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), mean cell volume (MCV), and mean cell hemoglobin concentration (MCHC). The RBC count determines the number of red blood cells per μl of whole blood, and the Hb reading determines the concentration of the protein hemoglobin in the blood. The hematocrit value is the percentage of whole blood occupied by cellular elements. This value is an approximate of the volume of erythrocytes as whole blood contains approximately 1000 red blood cells per white blood cell (Martini & Bartholomew). For this reason, the hematocrit value is often considered the packed cell volume (PCV). Another test, the mean cell volume (MCV) refers to the average volume of a single red cell. Lastly, the mean cell hemoglobin concentration (MCHC) determines the average amount of hemoglobin in one red blood cell (Martini & Bartholomew). A summary of these tests can be found in Table 2.1.

Table 2.1 Tests Relating to Red Blood Cells

TEST	WHAT IS MEASURED
Red Blood cell (CBC) count	# of RBCs per μl of whole blood
Hemoglobin (Hb)	Concentration of hemoglobin in the blood
Hematocrit (Hct)	% of formed elements in the blood
Mean Cell Volume (MCV)	Average volume of a single RBC
Mean Cell Hemoglobin Concentration (MCHC)	Average amount of Hb in one RBC

Adapted from Martini et al, 1997

B) *TESTS RELATED TO IRON STATUS: SERUM IRON, FERRITIN, TOTAL IRON BINDING CAPACITY:*

As previously mentioned, iron is essential for hemoglobin function, as oxygen forms a weak link with the iron ion contained within the heme molecule. As a result, approximately 70% of the iron present in the body is contained within circulating red blood cells. Other iron compounds include myoglobin (e.g. muscle hemoglobin), the cytochromes of the electron transport chain, and several Krebs-cycle enzymes, which help use oxygen at the cellular level (Williams, 2002). The remainder of the body's iron is either stored as ferritin in the liver or spleen, or circulating in blood plasma bound to the transport protein transferrin (Higgins, 2000). There are three tests to determine an individual's iron status: serum/plasma iron, serum ferritin, and total iron binding capacity (TIBC). The test for serum/plasma iron measures the concentration of the iron that circulates in blood plasma. It does not include the iron contained within hemoglobin, myoglobin or ferritin. The test for serum ferritin measures the concentration of ferritin in serum. This value reflects the total iron stores. The test for TIBC is essentially a measure of plasma transferrin concentration. These tests are summarized in Table 2.2.

Table 2.2 Laboratory Tests of Iron Status

TEST	WHAT IS BEING MEASURED
Serum/Plasma Iron	Plasma iron concentration.
Serum Ferritin	Concentration of ferritin in serum. Correlates with total body iron stores.
Total Iron Binding Capacity (TIBC)	Plasma transferrin concentration

Adapted from Higgins, 2000.

C) *APPROXIMATE REFERENCE RANGES:*

Reference ranges are laboratory and method specific, and may vary depending on age, sex, weight, diet or time of day (Nicoll et al, 2001). The reference ranges utilized in this study have been established for the Capital Health Region through cross-examination of the normal population (Dynacare Kasper Medical Laboratories, personal communication). The swimmers who participated in this study were males and females between the ages of 16 to 23 years. The Capital Health reference ranges for the research participants can be observed in Table 2.3.

Table 2.3. Approximate Reference Ranges for Research Participants

TEST	FEMALES	MALES
RBC	3.80-5.20 x 10^{12} L	4.30-6.00 x 10^{12} /L
Hb	120-160 g/L	135-175 g/L
Hct	0.36-0.46 L/L	0.41-0.52 L/L
MCV	80-100 fL	80-100 fL
MCHC	320-360 g/L	320-360 g/L
Iron	8-25 umol/L	8-25 umol/L
Ferritin	12-300 ug/L	12-300 ug/L
TIBC	40-80 umol/L	40-80 umol/L

Obtained from Dynacare Kasper Medical Laboratories, Edmonton, 2003

D) *EFFECTS OF EXERCISE ON RED BLOOD CELLS, HEMOGLOBIN AND HEMATOCRIT*

Numerous studies have demonstrated that physical exercise can influence hematological variables (Shaskey & Green, 2000; Schumacher et al, 2002). In these studies, comparisons have been made between athletes and their sedentary counterparts (Rietjens et al, 2002). As well, many investigators have examined the effects of endurance exercise on blood and iron variables (Schumacher et al, 2000; Schumacher et al, 2002). Recent research has also determined the effects of training workload on hematological

adaptations (Schumacher et al, 2002). The following sections review the current research into these areas.

1. Blood and Iron Status in Athletes vs. Sedentary Individuals:

Rietjens et al (2002) have reported that athletes as compared to non-trained individuals may show abnormal hematological values. Specifically, researchers have stated that athletes from various sporting disciplines exhibit decreased RBC, Hct and Hb levels compared to physically inactive controls (Schumacher et al, 2002; Rietjens et al). However, some authors argue that these changes are not dependent on physical activity itself, but rather on the specific type of exercise, such as endurance training. For instance, Schumacher et al, examined 851 males (747 athletes, mean age 24.2 years; 104 controls, mean age 29.9 years). The athletes were subdivided into 3 categories; endurance athletes such as cycling and running (n=426); strength trained athletes such as weight lifting and discus (n=203); and mixed athletes such as soccer and volleyball players (n=118). The researchers found that there was no difference between hemoglobin and hematocrit levels in the inactive controls compared to the physically active subjects in general, but the endurance trained athletes experienced lowered Hb, RBC, and Hct levels compared not only with sedentary individuals, but also with strength-trained athletes. Further studies need to be conducted to clarify differences in hematological variables for athletes in different sports.

Other studies have demonstrated that iron and especially ferritin levels have been reduced in athletes compared to sedentary controls (Schumacher et al, 2002; Rietjens et al, 2002). Shaskey & Green (2000) reviewed the literature on anemia in athletes. The studies

reviewed postulated varying reasons for reduced iron levels in athletes, including increased loss through sweat, the gastrointestinal (GI) tract, and the genitourinary (GU) tract. The GI and GU tracts may be sources of blood loss following intense endurance exercise. It has also been shown that hematuria can occur after exercise even in non-contact sports (Shaskey et al). Despite this, Schumacher et al found that the ferritin and iron levels of the athletes in their study were still within the normal range.

2. Effects of Endurance Exercise on Hematological Measures:

Hematological changes associated with endurance training have been extensively studied. Numerous authors have reported that the reduced RBC, Hb and Hct values seen in endurance athletes are mainly due to exercise-induced plasma volume expansion (Schumacher et al, 2002; Shaskey et al 2000; O'Toole et al, 1999). The exact mechanism for the increased plasma volume is not completely understood. It has been postulated by many researchers that an increased plasma volume can increase exercise capacity by reducing blood viscosity thereby allowing greater cardiac output and greater overall oxygen delivery (Shaskey et al; Schumacher et al).

However, this is disputed by other researchers who claim that decreased RBC, Hb and Hct levels are actually detrimental to athletic performance. Studies have shown that higher Hb levels are correlated with improved performance (Shaskey & Green, 2000). These authors contend that decreased Hb, RBC and Hct levels are side effects of an intense and heavy workload (Shaskey & Green). Therefore, more research into this area is required.

Nonetheless, it seems clear that Hb, RBC and Hct levels are lower in exercising populations compared to non-exercising populations. In addition, this difference appears to

be greatest when examining elite, endurance athletes compared to control individuals (Shaskey et al, 2001).

3. Hematological Values Related to Training Workload and Level of Performance:

The decrease in Hb, RBC and Hct levels in endurance athletes has also been attributed to training workload. Studies have demonstrated that changes in Hb, RBC and Hct levels are related to exercise duration and intensity (Schumacher et al, 2000; Rietjens et al, 2002). For instance, Mackinnon et al (1997), observed the hematological responses to a 4-week progressive increase in training volume and intensity in 8 male and 16 female competitive swimmers (aged 15-26 years) and found that erythrocyte numbers, Hb concentration, and Hct levels declined significantly over the 4 weeks. Similarly, Rietjens et al found hematological values that were below the lower limit of the normal range in 46% of the athletes during the off-season, in 55% of the athletes during the training season, and in 72% of the athletes during the racing season. This study observed 11 triathletes (7 males and 4 females, mean age 26.4 years) over a three-year period. This progressive decline in the hematological variables with increasing training volume and/or intensity may be attributed to increased plasma volume. According to Shaskey & Green (2000), the degree of plasma volume expansion correlates with the amount and intensity of exercise, so that with hard training, elite endurance athletes exhibit the greatest plasma volume increase. It is significant that a restoration of Hb and Hct levels has been observed with various tapers which involve a reduction in training volume (Houmard & Anderson Johns, 1994). This is critical as an increase in Hb and Hct levels prior to competitions may enhance oxygen carrying capacity and physical performance (Houmard & Anderson Johns).

IMMUNOLOGICAL MEASURES:

The immune system is influenced by a range of physical, psychological, behavioural and environmental factors (Pyne et al, 2000a). Thus, a physical stress such as exercise may have many profound effects on an individual's immune function. Numerous researchers have demonstrated that exercise alters various immune parameters leading to either enhanced or suppressed immunity (Gleeson et al, 1995; Pyne et al; Jonsdottir, 2000). The nature of the immune changes depends on a variety of factors including the immune parameter of interest; volume, intensity and mode of exercise; fitness level; environmental factors; and the time course of measurement (Nieman & Klarlund, 2000). For instance, it is widely accepted in the current literature that there is a "J-curve" relationship between exercise and upper respiratory tract infections (URTI). This model suggests that moderate exercise may reduce susceptibility to illness, but the risk may increase progressively during periods of excessive amounts of high-intensity exercise (Neiman & Klarlund). Divisions of the immune system which have been identified by researchers to change with exercise include: the distribution of leukocyte (white blood cell) subsets, concentrations of lymphocyte subsets, functional activities of natural killer cells and neutrophils, and concentration of antibodies or immunoproteins such as salivary immunoglobins (Pyne et al). As well, exercise immunology literature has examined other factors that might influence cellular function or release of soluble factors such as glutamine or glutamate (Nieman & Klarlund).

The next section provides a brief overview of the immune system, followed by a discussion of the composition and function of the white blood cell system and it's constituents, and the amino acids glutamine and glutamate. As well, the effects of exercise on the aforementioned parameters are reviewed.

A) *OVERVIEW OF THE IMMUNE SYSTEM:*

The immune system is the body's defense system, as it is capable of recognizing and defending the body against foreign invaders such as viruses, bacteria, fungi, parasites and allergens (Mackinnon, 1999). The immune system can be divided into two major systems: innate and acquired immunity.

Innate immunity is defined as the immune defenses that are present early in life. Generally, these defenses do not require previous exposure to a particular pathogen, and therefore they are the first line of defense (Field et al, 2000). The innate immune response includes physical barriers (e.g. skin and mucous membranes), and cellular components (e.g. phagocytes and natural killer cells) (Field et al).

In contrast, acquired immunity develops specifically against individual invading agents. This specific response is developed through prior exposure to the pathogen. The acquired response creates a memory of each encounter with a foreign agent, thus allowing a more efficient and quicker response to a repeated exposure (Mackinnon, 1999). The acquired immune system consists of humoral immunity and cell-mediated immunity (Viru & Viru, 2001). The humoral immune system consists of B-lymphocytes that form antibodies (e.g. immunoglobins). The cell-mediated immune system consists of two types of T-lymphocytes, CD4 (helper) and CD8 (suppressor) cells.

While both the innate and the acquired immune systems have different responses to a pathogen, they both work together. Thus, both are very important for the overall functioning of the body's immune system.

B) *COMPOSITION AND FUNCTION OF IMMUNE PARAMETERS:*

1. White Blood Cells (Leukocytes) and Differential:

White blood cells, also known as leukocytes, play a major role in the immune system (Mujika et al, 1996a). Specifically, leukocytes constitute a mobile part of the body's inflammatory response (Viru & Viru, 2001). The purpose of this response is to ensure a rapid defense against infectious agents, to contain and control injury, and to initiate healing and tissue repair (Higgins, 2000). The white blood cells are actually comprised of five distinct populations (neutrophils, monocytes, basophils, eosinophils and lymphocytes) which each have a different function. Table 2.4 summarizes the function and proportion of total leukocytes for each of the five populations.

Table 2.4 Summary of White Blood Cells Subsets and Functions

WHITE BLOOD CELL	FUNCTION	PREVALENCE IN HUMAN PERIPHERAL BLOOD
Neutrophils	Usually the 1st to arrive at an injury site. Are active phagocytes (engulf pathogens or debris in tissues).	50-70% of leukocytes
Monocytes	Phagocytose and kill foreign organisms; present antigens to T-lymphocytes	1-10% of leukocytes
Basophils	Release histamine and other chemicals that decrease inflammation in damaged tissues.	0-1% of leukocytes
Eosinophils	Attack foreign material too large for normal phagocytosis (protect against infection by organisms larger than bacteria and viruses).	0-3% of leukocytes
Lymphocytes	Initiate immune response; produce antibodies; cytokine production; remember invading organisms.	20-40% of leukocytes

Adapted from Martini & Bartholomew (1997); Viru et al (2000); Nieman & Klarlund (2000).

a. *Interpretation of White Blood Cell and Differential Test Results:*

Values for white blood cells and the five distinct populations can be obtained through a complete blood count (CBC) and differential. The total white cell count is the sum

of all the white cell types, while the differential white cell count is a count of each of the five populations (neutrophils, monocytes, basophils, eosinophils, and lymphocytes) (Higgins, 2000). The reference ranges, determined by the Capital Health Region, provided below are based on the age and gender of the participants in this study (Table 2.5).

Table 2.5 Approximate Reference Ranges for White Blood Cells and Differential

TEST	FEMALES	MALES
Total White Blood Cell Count	4.0-11.0 x 10^9 /L	4.0-11.0 x 10^9 /L
Neutrophils	1.8-7.5 x 10^9 /L	1.8-7.5 x 10^9 /L
Lymphocytes	1.0-4.5 x 10^9 /L	1.0-4.5 x 10^9 /L
Monocytes	0.0-1.1 x 10^9 /L	0.0-1.1 x 10^9 /L
Eosinophils	0.0-0.7 x 10^9 /L	0.0-0.7 x 10^9 /L
Basophils	0.0-0.3 x 10^9 /L	0.0-0.3 x 10^9 /L

Obtained from Dynacare Kasper Medical Laboratories, Edmonton

2. Lymphocytes (T-cells, B-cells, and Natural Killer Cells):

There are three classes of lymphocytes, each with different functions. The three types of lymphocytes are: T-lymphocytes, B-lymphocytes, and natural killer (NK) cells. T-cells develop in the thymus, and account for approximately 75% of lymphocytes (Adams & Kirkby, 2001). T-cells can also be classified into three groups: helper T-cells, cytotoxic T-cells, and suppressor T-cells (Viru & Viru, 2001). Helper T-cells (CD4+) stimulate the activities of both the T-cells and B-cells, cytotoxic T-cells (CD8+) attack cells directly, and suppressor T-cells inhibit both T-cells and B-cells (Martini & Bartholomew, 1997). B-lymphocyte cells, on the other hand, are formed in the bone marrow, and make up approximately 10% of total lymphocytes. B-cells, when activated to plasma cells, produce five immunoglobin subclasses: IgM, IgG, IgA, IgD and IgE (Adams & Kirkby, 2001). Immunoglobins are glycoproteins that appear in serum and secretions. The remaining lymphocytes are natural killer (NK) cells, which are larger than most resting T- and B-

lymphocytes. NK cells are functionally different from T- cells in that they lack immunological memory, do not carry the T-cell surface marker CD3, and do not require maturation in the thymus (Jonsdottir, 2000). NKcells (CD16+CD56+) destroy a number of target cells including tumor cells, cancer cells, and normal cells that are infected with viruses (Jonsdottir; Martinin & Bartholomew). (Note: leukocytes display unique cell surface antigens which are used to identify the various subsets. The cell surface proteins are identified by the prefix CD which stand for cluster of differentiation) (Nieman & Klarlund, 2000).

3. Salivary Immunoglobin A (S-IgA):

As mentioned above, immunoglobins are produced from mature B-lymphocytes, and are glycoproteins that appear in serum and secretions (e.g. saliva, tears). An immunoglobin that reacts with a specific antigen is termed an antibody. An antibody stimulates other immune cells to kill the foreign pathogen (Nieman & Klarlund, 2000). Immunoglobins exist in five subclasses, however the literature in exercise immunology has focused mainly on IgA (Nieman & Klarlund). IgA is found in glandular secretions such as tears, mucus and saliva. The primary function of IgA is to bind to pathogens before they enter the body tissues (Martini & Bartholomew, 1997).

4. Plasma Glutamine:

Glutamine is the most abundant amino acid in the body (Castell & Newsholme, 2001). It can be synthesized in the body and therefore is classified as a non-essential amino acid (Castell et al, 1996). Glutamine is an important energy source for cells of the immune

system, particularly lymphocytes and macrophages (Nieman et al. 2000). Lymphocytes and macrophages utilize glutamine at a rate that is similar to, or even greater than that of glucose (Castell & Newsholme, 2001). This suggests that a decrease in plasma glutamine concentration below physiological levels may impair the function of the cells of the immune system (Castell et al, 1996). As well, glutamine fulfils many other roles in the body including transfer of nitrogen between organs and detoxification of ammonia, a fuel for gut mucosal cells, and maintenance of acid-base balance during acidosis.

a. Glutamine Metabolism:

Glutamine is used at a high rate each day. Exchange rates of glutamine exceed the body's total stores of glutamine by several fold each day (Rowbottom et al, 1996). Therefore, there is a constant need for glutamine synthesis in the body (Rowbottom et al). The major organs of glutamine synthesis include: skeletal muscle, liver, lungs, heart and brain (Nieman et al, 2000, and Rowbottom et al). Skeletal muscle is the most important site for glutamine synthesis (Nieman et al). At the intracellular level, glutamine is produced from glutamate and ammonia catalyzed by the enzyme glutamine synthetase (Nieman et al). This reaction is discussed in more detail in the next section.

5. <u>Plasma Glutamate:</u>

The non-essential amino acid glutamate (also known as glutamic acid), plays a key role in muscle energy metabolism, and is therefore an important amino acid to analyze with regards to overtraining. The intermediary metabolism of several amino acids including

glutamate, alanine, and the branched chain amino acids (BCAA), affects other metabolites, notably the intermediates within the tricarboxylic acid (TCA) cycle (Gibala, 2000).

a. Glutamate Metabolism:

Specifically, glutamate appears to be a key substrate for the synthesis of muscle TCA cycle intermediates (such as 2-Oxoglutarate) as well as the amino acid glutamine (Wagenmakers, 1992). This involves two reactions:

1.) Glutamate + Pyruvate ↔ Alanine + 2-Oxoglutarate

2.) Glutamate + ammonia + ATP ↔ Glutamine + ADP

In the first reaction, glutamate may donate the amino group to pyruvate to form alanine and regenerate 2-Oxoglutarate (Wagenmakers, 1992). The carbon skeletons for the synthesis of alanine are likely derived from muscle glycogen or glucose taken up from the circulation (Wagenmakers) (Figure 2.2). This reaction can also be reversed. In this case, an amino group will be donated to 2-Oxoglutarate to form glutamate.

In the second reaction, glutamate reacts with ammonia to form glutamine (Figure 2.2). This reaction is catalyzed by the enzyme glutamine synthetase (Wagenmakers, 1992). The carbon skeletons for the synthesis of glutamine may be derived from muscle glycogen and blood glucose, as well as from degradation of valine and isoleucine to TCA cycle intermediates (Wagenmakers). The synthesis of glutamine is important for several reasons, as discussed previously.

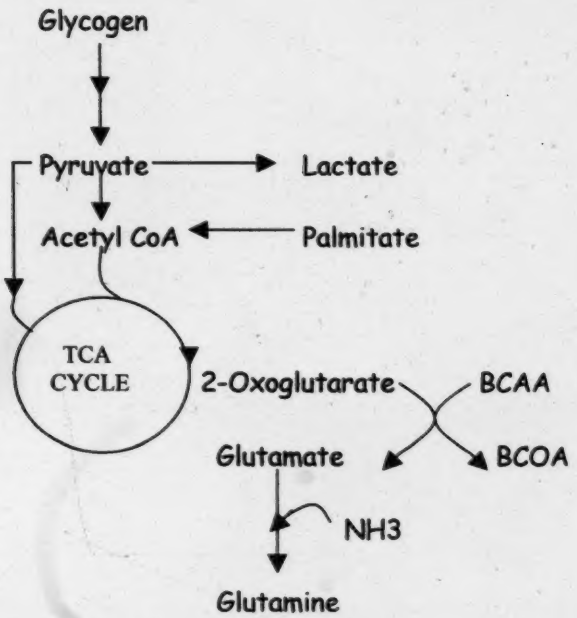

Figure 2.2. Glycogen may act as a Precursor for the Synthesis of the Carbonskeletons of Glutamate and Glutamine. Adapted from Wagenmakers (1992).

C) *EFFECTS OF EXERCISE ON IMMUNE PARAMETERS*:

As previously mentioned, exercise affects a variety of immune parameters. The immune response associated with exercise stress involves the coordination of many cell types, soluble factors, and messenger molecules in the blood and throughout the body (Venkatraman & Pendergast, 2002). The alterations to the various immune parameters with exercise may give rise to either suppressed or enhanced immunity (Gleeson et al, 1995). Researchers have demonstrated that regular and moderate exercise improves the ability of the immune system to protect against infection (Venkatraman & Pendergast; Pyne et al, 2000a). However, there is a fine line between training that improves immunity and training that suppresses immunity. Many studies have shown that if exercise is chronic and/or severe (high volume and intensity), an individual's immunity will be suppressed, and his or her

vulnerability to infection or illness will be increased (Venkatraman & Pendergast; Mackinnon, 1997; Pyne et al). To date, several models have been developed to explain this concept. The "J-shaped" model suggests that individuals who exercise moderately exhibit a lower incidence of upper respiratory illnesses (URTI), while athletes undertaking strenuous training exhibit increased incidence of infection (Mackinnon). This has led to the "Open Window" hypothesis, which suggests that there is a time period after intensive exercise during which an athlete is at increased risk of infection (Mackinnon). This review examines the factors which influence immunocompetence in athletes including: leukocyte number, NK cells, T- lymphocytes, IgA, and the amino acids glutamine and glutamate.

1. Leukocyte Number:

Resting blood leukocyte numbers are generally clinically normal in athletes (Mackinnon, 2000). Studies have demonstrated that exercise induced changes in leukocyte numbers are transitory in that they change with acute exercise, but usually return to normal levels within 12 to 24 hours post exercise (Nieman & Klarlund, 2000). However, some researchers have demonstrated chronic effects of exercise on leukocyte numbers, although the results are equivocal. Some athletes who undergo prolonged periods of very intense training have shown clinically low leukocyte numbers. For example, Lehmann et al (1996) found that leukocyte numbers progressively declined throughout 4 weeks of intensified training in male runners. In contrast, other studies have reported no significant differences in leukocyte numbers between well-trained and overtrained athletes (Mackinnon; Hooper & Mackinnon, 1995). For instance, Hooper et al found no differences in the leukocyte number of well-trained elite competitive swimmers and overtrained swimmers

during a 6-month competitive season. Fourteen swimmers (5 male and 9 female, mean age 17.4 years) were monitored for physiological and mood-state changes over the season. Three swimmers were classified as overtrained based on performance deterioration and prolonged high fatigue. Their leukocyte levels were not significantly different from the remaining 11 swimmers. Therefore, more research into this area is needed.

2. Natural Killer (NK) Cells:

Resting natural killer (CD16+CD56+) cell numbers in the peripheral blood are generally normal in athletes (Mackinnon, 2000). It has been well documented however that acute exercise is associated with changes in NK cell numbers. Researchers have shown that NK cell numbers increase during exercise, but decrease immediately after (Gleeson et al, 2000; Nieman & Klarlund, 2000; Jonsdottir, 2000). More specifically, NK cell numbers have been reported to increase up to three times resting levels during exercise, but fall below baseline values 1 to 6 hours post exercise (Nieman et al).

The chronic effects of exercise training on NK cell numbers have also been well documented. Research has demonstrated that NK cell numbers may decrease during short term and long term intense training (Mackinnon, 2000; Gleeson et al, 2000). For example, two different studies which both examined competitive swimmers found NK cell numbers to decrease throughout the training period. Gleeson et al reported that the intensive exercise of a 12 week training program was associated with a decline in NK cell numbers in 22 elite swimmers (12 males and 10 females) aged 16-22 years. Similarly, Gleeson et al (1995), found that NK cell numbers declined by 30% -40% after 7 months of intense swim training in 26 elite swimmers (15 males and 11 females) aged 16-24 years. The biological significance of

the changes in NK cell numbers has not yet been identified (Mackinnon). It is speculated that a fall in NK cell numbers, whether acutely or chronically, may leave an athlete susceptible to viral infections and illness (Gleeson et al, 2000).

3. T-Lymphocytes:

Peripheral blood T-lymphocytes are typically within normal ranges in athletes (Mackinnon, 2000). Research has shown T-cell numbers to increase with acute exercise. Specifically, CD4+ (helper/inflammatory) and CD8+ (cytotoxic/suppressor) T-cell concentrations increase with exercise. However, the ratio of CD4+ to CD8+ generally declines due to the fact that CD8+ cells increase to a greater extent than CD4+ cells. These changes do not persist for long periods of time as CD4+ and CD8+ cells both tend to decline below pre-exercise values 1 - 4 hours post exercise (Nieman & Klarlund, 2000). However, researchers have suggested that a ratio of CD4+ to CD8+ cells below 1.5 is subnormal, and may be a cause and an indicator of immunosuppression (Castell & Newsholme, 2001).

4. Salivary Immunoglobulin A (S-IgA):

Of the five immunoglobin subclasses, exercise immunology literature has focused mainly on IgA in mucosal (saliva) secretions (Nieman & Klarlund, 2000). This is because the incidence of upper respiratory tract infection (URTI) in athletes has been frequently related to the level of salivary IgA (S-IgA) (Krzywkowski, Petersen, Ostrowski et al, 2001; Nieman et al). Coaches and athletes associate overtraining with frequent illness, especially URTI (Mackinnon, 2000). Symptoms of URTI include running nose, nasal congestion, and

sore throat. The incidence of URTI appears to be highest after a major competition or during prolonged or intensive training (Nieman et al). Several researchers have demonstrated that S-IgA levels decrease after prolonged or intense exercise (Krzywkowski et al; Gleeson et al, 1995). This plays an important role in immunity as resistance to respiratory infection is provided by the mucosal immune system, with the main immunoglobulin being S-IgA (Krzywkowski, Petersen, Ostrowski et al). Therefore, a decrease in S-IgA may be associated with URTI and /or overtraining.

5. Plasma Glutamine:

According to Rowbottom et al (1996), the amino acid glutamine may have a significant role in monitoring training. Glutamine has been suggested as an indicator of a return to health. In a situation of exercise stress, plasma glutamine levels may be useful for indicating an overtraining state, and also for monitoring the recovery of an athlete experiencing performance decrements (Rowbottom et al). Following short-term exercise, the concentration of plasma glutamine has been reported to increase (Castell et al, 2000). However, several studies have shown that plasma glutamine levels decreased following prolonged or intensive training sessions (Nieman & Klarlund, 2000). The decrease in plasma glutamine is due to net over-utilization by the liver, kidneys, or cells of the immune system. In overtrained athletes, studies have shown that plasma glutamine levels have been significantly lowered (Rowbottom et al, Nieman et al). The decreases in plasma glutamine following exercise stress may be sufficient to increase an athlete's susceptibility to infection (Rowbottom et al). Therefore, glutamine may be a useful immunological measure.

6. Glutamine / Glutamate Ratio:

Smith & Norris (2000), hypothesized that the ratio between glutamine and glutamate concentrations may be used as a marker of overtraining in athletes. They studied 52 Canadian national team athletes (31 male and 21 female) from various sports including speed-skating, swimming and cross-country skiing over 2 - 4 macrocyles. Based on the results of their study, Smith & Norris proposed that with an increased training load, glutamine levels will decrease and glutamate levels will increase. Parry-Billings & Budgett (1992) also reported a decreased glutamine concentration, and an increased glutamate concentration in overtrained athletes. The changes in the concentrations of these amino acids may be very significant. The ratio of glutamine to glutamate may globally represent overall tolerance to training (Smith & Norris). Specifically, it is speculated that glutamine concentration may reflect an individual's tolerance to volume of work, and glutamate concentration may reflect tolerance to high intensity training (Smith and Norris). However, more research is needed in this area.

D) SUMMARY OF IMMUNOLOGICAL MEASURES:

Based on the present review of literature into the area of exercise immunology, it is clear that there are a number of parameters that may be useful in the monitoring of training in elite athletes. The serial monitoring of athletes throughout a training season provides coaches and medical personnel with feedback that may be useful in preventing performance decrements, and in reducing the risk of illness that compromises training.

HORMONAL MEASURES: CORTISOL

Over the past 30 years, numerous advancements have been made in the area of exercise endocrinology. Research into this area has contributed greatly to the understanding of the physiological adaptations that occur with exercise and physical training (Tremblay & Chu, 1994). For instance, various studies have demonstrated altered hormone responses in highly trained and overtrained athletes (Mujika et al, 1996b).

A number of stress hormones have been monitored in an attempt to understand possible mechanisms and to find reliable indicators of overtraining (Hooper et al, 1993). One such hormone, which has been the focus of the majority of research in this area, is cortisol. Thus, cortisol has been proposed as a marker to monitor the stress of training and to evaluate training responses in order to avoid overtraining in athletes (Mujika et al, 1996b).

The purpose of this section is to examine the major physiological roles of cortisol and to explore the normal response of cortisol to exercise. More importantly, this review examines the response of cortisol to overtraining, the mechanisms which underlie the physiological changes, as well as the advantages and disadvantages of these adaptations.

A) PHYSIOLOGICAL ROLES OF CORTISOL:

1. Cortisol Production and Feedback:

The hormone cortisol is an adrenal steroid hormone. Cortisol is produced by cells in the adrenal cortex, and is regulated by anterior pituitary Adrenocorticotropic hormone (ACTH). The release of ACTH is in turn regulated by hypothalamic Corticotropin-releasing hormone (CRH). Cortisol participates in a negative feedback loop along with ACTH. This

feedback is mediated at the level of the pituitary, hypothalamus, and even the central nervous system (CNS) (Hadley, 1996). This association is referred to as the hypothalamic-pituitary-adrenal (H-P-A) axis (Figure 2.3).

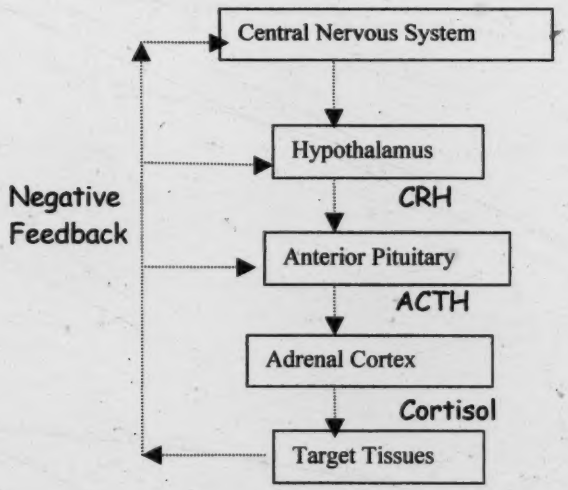

Figure 2.3. The Hypothalamic-Pituitary-Adrenal Axis (H-P-A)

2. Major Physiological Roles of Cortisol:

Cortisol has a wide spectrum of metabolic effects and therefore influences the control of several metabolic pathways (Viru & Viru, 2001). Specifically, major functions of cortisol include the stress response, anti-inflammatory effects, immunosuppressive actions, and intermediary metabolism.

Cortisol plays a major role in the stress response and is justifiably called a stress hormone. In various stressful situations the cortisol concentration in the blood increases sharply. Examples of stressful situations include exercise, trauma, infections, illnesses, and emotional stain (Viru & Viru, 2001). As well, cortisol aids in the stress response because an increase in cortisol causes an increase in the synthesis of the catecholamines, epinephrine

and norepinephrine. More specifically, cortisol maintains vascular reactivity, which means that without cortisol, blood vessels are unable to respond to circulating catecholamines (Hadley, 1996).

Cortisol also has an anti-inflammatory effect. Damaged tissue usually becomes inflamed, but in certain cases, the inflammation is more damaging than the trauma or disease itself (Viru & Viru, 2001). Therefore, cortisol decreases inflammation by inhibiting the inflammatory reaction (the normal response of tissues to injury), and thereby avoiding the harmful effects of exaggerated inflammation (Hadley, 1996). Thus, cortisol is administered in pharmacological doses as an anti-inflammatory.

In addition, cortisol suppresses the immune system and excess cortisol causes atrophy of the lymphatic system. For example, this can result in failure of the body to provide antibodies during an infection. Specifically, the concentration of lymphocytes and eosinophils in blood decreases under the influence of high cortisol (Viru & Viru, 2001). This immunosuppressive action may be helpful in avoiding the unwanted effects of local infections (e.g. an exaggerated inflammation response). However, it may also be very harmful as it leaves the individual susceptible to bacterial infections (Hadley, 1996).

A further metabolic effect of cortisol is in relation to intermediary metabolism. An increase in cortisol stimulates gluconeogenesis, which leads to elevated blood glucose levels. Specifically, cortisol causes proteolysis of muscle proteins, and lipolysis of fat to occur. The free fatty acids and amino acids that are then released from these tissues become available as substrates for gluconeogenesis within the liver. This results in an increased synthesis of glucose (Hadley, 1996). See Figure 2.4 for a diagram on this process.

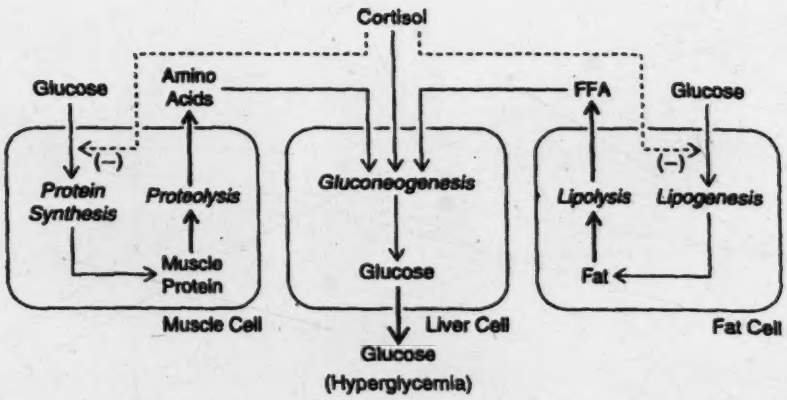

Figure 2.4. Major Effects of Excess Cortisol in Intermediary Metabolism
(Taken from Hadley, 1996)

B) *NORMAL RESPONSE OF CORTISOL TO EXERCISE:*

Based on the discussion regarding the physiological roles of cortisol, it is clear that exercise is considered a stressful situation to the body, and therefore an increase in cortisol occurs along with numerous other metabolic effects. Specifically, the H-P-A axis is modulated by exercise, and participates in the maintenance of homeostasis within the body. Physical exercise presents a challenge to homeostasis (Luger et al, 1987) and therefore, increasing levels of cortisol help to maintain the body's homeostasis during exercise. However, this rise in cortisol is dependent upon the intensity and duration of the exercise, as well as the physical fitness level of the individual (Luger et al).

Firstly, the intensity of the exercise correlates well with the cortisol level, in that light to moderate workloads produce little increase in cortisol from basal levels, whereas with moderate to heavy workloads there is a progressive rise in cortisol levels. Luger et al. (1987), found that exercise at 50% of VO_2 max in 7 untrained (mean age 35.7 years), 7 moderately trained (mean age 30.0 years) and 7 highly trained (mean age 31.6 years) male runners caused no elevation in plasma ACTH or cortisol levels. However, exercise at 70%

and 90% of VO_2 max was associated with a proportional activation of the H-P-A axis in all subjects (Luger et al). Therefore, the intensity-dependent increase in adrenal cortisol was stimulated by an increase in pituitary ACTH.

An increase in cortisol levels is also related to the duration of exercise. Short-term exercise causes little alteration in cortisol levels. Prolonged exercise however can cause elevations in cortisol far above basal levels. A significant increase in blood cortisol concentrations usually requires a duration of exercise of more than 20 minutes (Warren & Constantini, 2000).

Physical fitness also affects cortisol levels. According to Fry et al (1991b), training may be able to facilitate an adaptation to stress because gradually increasing training loads have been shown to increase the stability of the H-P-A axis. Further, a study by Luger et al (1987), demonstrated that physically fit male runners (mean age 31.6 years) experienced much less activation of the H-P-A axis compared with untrained males (mean age 35.7 years) at matched absolute workloads. Therefore, adaptation to regular aerobic exercise is related to a reduced response in the H-P-A axis. Such an adaptation results in an individual being able to handle a higher workload with less pituitary-adrenal activation (Luger et al).

To summarize, an acute exercise training session appears to cause an increase in cortisol levels. The magnitude of the increase is dependent on a variety of factors including intensity and duration of exercise as well as the fitness level of the individual. However, this is merely an examination of the acute effects of regular aerobic exercise and does not explain the adaptations that occur with overload training. The next section examines the effects of overload training on cortisol levels in endurance athletes. Overload training

refers to a planned, systematic and progressive increase in training stimuli which is necessary for improved performance (Armstrong & Van Heest, 2002).

C) *CORTISOL RESPONSE TO OVERLOAD TRAINING:*

Improvements in an athlete's performance are achieved through training, which induces physical, physiological, and psychological stress. Applying a series of stimuli will displace the homeostasis of the athlete's functional systems, providing a stimulus for adaptation (Fry et al, 1991b). This is achieved through overload training, which was described previously. Numerous studies have suggested that hormonal adaptations occur with overload training. Table 2.6 summarizes the findings from studies which have examined the cortisol levels in athletes who have undergone a dramatic increase in training volume and/or intensity, but who are considered to be optimally trained.

Table 2.6. Studies involving cortisol levels of athletes that are optimally trained.

REFERENCE	STUDY DESIGN	FINDINGS
Kirwan et al (1988)	-12 M swimmers (mean age 19.1 yrs) -doubled training volume while maintaining intensity at 95% VO_2 max for 10 days	- serum CORT sig. ↑ but performance not impaired. - suggest normal response of ↑ training load
Mujika et al (1996b)	- 8 highly trained male swimmers (mean age 21.1 yrs) - 12 weeks intense training & 4 weeks taper	No statistically sig. changes in plasma [CORT] during training and taper
Costill et al (1991)	- 24 M swimmers into 2 groups: (6 weeks) LONG – 2 workouts/ day for 12 M (mean age 19.2 yrs) SHORT – one workout/day for 12 M (mean age 19.6 yrs)	Serum CORT sig. ↑ as consequence of ↑ training with LONG group.
Bonifazi et al (2000)	- GROUP 1: 8 M swimmers (aged 19-25 yrs) 18 week study - GROUP 2: 10 M swimmers (aged 18-22 yrs) 14 week study	- Both groups: ↑ in rest plasma CORT at end of 4 week period of higher training volume - ↓ in plasma CORT during taper (suggest prerequisite for improved performance)

Based on the review of literature in this area, the results are somewhat equivocal. Several researchers have suggested that cortisol levels increase with overload training (Kirwan et al, 1988; Costill et al, 1991; Bonifazi et al, 2000), however some researchers believe that cortisol levels do not change (Mujika et al, 1996b).

Kirwan et al (1988), examined 12 male swimmers (mean age 19.1 years) for a 10 day training camp in which the training volume was doubled while the training intensity was maintained at 95% of VO_2 max. The results of this study demonstrated that cortisol levels were significantly increased due to the increase in training volume. The swimmers in this study did not experience performance decrements, therefore the authors suggested that the increase in cortisol was a normal response to the increased training load. Similarly, Costill et al (1991) found that cortisol levels increased as a consequence of an increased training volume. In this study 24 male swimmers were divided into 2 groups. One group (12 males, mean age 19.6 years) swam only once per day while the second group (12 males, mean age 19.2 years) swam twice per day for 6 weeks. Based on the results of the study, only the group that swam twice per day experienced an increase in cortisol levels. Interestingly, the athletes in both of the groups experienced performance improvements at the end of the 6 weeks.

However, research has also shown that cortisol levels do not change with a significant increase in training volume and intensity. Mujika et al (1996b), examined 8 highly trained male swimmers (mean age 21.1 years) during 12 weeks of intense training and 4 weeks of tapering. These authors found that there were no statistically significant changes in cortisol concentrations during training and tapering.

Conversely, a study by Bonifazi et al (2000), which also examined the differences in cortisol between intensive training and tapering found differing results. Bonifazi et al, examined 2 groups of male swimmers for 14 to 18 weeks (group 1 was 8 males aged 19-25 years; group 2 was 10 males aged 18-22 years). In both of these groups an increase in resting cortisol was observed at the end of a 4-week period of higher training volume. Interestingly, these authors also found a decrease in cortisol levels with tapering. Bonifazi et al suggested that a decrease in cortisol was possibly a prerequisite for improved performance.

It is important to realize, however, that the varying findings of these studies may be a result of methodological inconsistencies. For instance, the choice of sample (e.g. serum vs. plasma) used in the cortisol determination may have impacted the results. As well, the length of the study, and the method of inducing overload training may lead to different results. For instance, the study by Kirwan et al (1988) was only 10 days in duration, whereas the study by Bonifazi et al (2000) was 18 weeks. Although the exact implication of these methodological inconsistencies is unknown, it is important to realize that the results may be confounded.

Despite the varying findings in these studies, and the possible methodological limitations, it appears as though an increase in cortisol due to intensive overload training is the most common conclusion among researchers. Therefore, this suggests that an increase in cortisol levels is a normal response to a dramatically increased training load. However, based on the above literature review, it also appears as though the hormonal alterations that occur with overload training appear to be fairly transient. In well-trained athletes, tapering or a decrease in training load has been shown to decrease cortisol levels.

This leads to the question as to whether overtraining causes more chronic adaptations in the neuroendocrine system. The following section reviews the effects of cortisol in response to overtraining.

D) *CORTISOL RESPONSE TO OVERTRAINING:*

The concentration of the stress hormone cortisol has been shown to change with overtraining (Uusitalo, 2001). Unfortunately, there have been discrepant findings regarding resting cortisol levels. Some studies have reported increases, decreases, or no change in cortisol levels (Urhausen & Kindermann, 2002).

Table 2.7. Studies involving cortisol levels of athletes in a state of overtraining

REFERENCE	STUDY DESIGN	FINDINGS
INCREASED CORT:		
O'Connor et al (1989)	- 14 F college swimmers; 5.5 month study - 3 in OT - training volume +600% over 3 weeks	Saliva CORT ↑
Luger et al (1987)	- 21 M: 7 untrained (aged 35.7 ± 0.9), 7 mod. trained (24-40 Km/wk) (aged 30.0 ± 3.4), 7 highly trained runners (>75 Km/wk) (aged 31.6 ± 2.6)	Resting plasma CORT ↑ in highly trained compared to untrained or mod. trained
Barron et al (1985)	- 9 M marathon runners, aged 22-36 years; 4 months of intensive training; 4 in OT	OT athletes had ↑ basal plasma CORT levels
UNCHANGED CORT:		
Hooper et al (1993)	- 5M, 9 F swimmers (mean age 17.2 ± 1.5 years); 6 month study; 3 in OT	No sig. diff. between OT and well-trained in plasma CORT levels across training period
Mackinnon et al (1997)	- 8 M, 16 F swimmers (aged 15-26 years) - 4 week study - training volume +37%	No changes in plasma CORT
Urhausen et al (1998)	- 17 M endurance athletes (mean age 23.4 years) (cyclists and triathletes) - 19±1 month study	Resting CORT during OT comparable to normal training states.
DECREASED CORT:		
Lehmann et al (1992)	- 8 M mid and long distance runners (mean age 33.7 years); 5 week study - training volume +100% over 3 weeks	Resting plasma CORT ↓

Numerous studies have shown that resting cortisol levels increase during overtraining, or with excessive increases in training volume or intensity (Hooper et al, 1993; Costill et al, 1991; Uusitalo, 2001). In some highly trained athletes, who may be considered overtrained, there is a dramatic increase in cortisol levels resulting in a condition known as hypercortisolism (Luger et al, 1987). This hypercortisolic state has also been seen in individuals with amenorrhea, anorexia nervosa, and/or depression (Luger et al). For instance, Barron et al (1985) found that 4 overtrained male marathon runners (ages 24, 24, 26 and 35) had elevated basal cortisol levels compared to 5 other optimally trained male marathon runners (aged 22 -36 years) during a 4 month study.

Alternatively, other researchers have reported normal basal cortisol concentrations in overtrained athletes, or cortisol levels that have not changed following intensified training (Hooper et al, 1993; Mackinnon, 1997). For instance, both Hooper et al, and Mackinnon, who examined the hormonal responses of elite swimmers to overtraining, found no significant differences between the resting cortisol level in overtrained swimmers compared to well-trained swimmers. As another example, Urhausen et al (1998), found that resting cortisol concentrations during overtraining were comparable to normal training states. This study examined 17 male endurance athletes (mean age 23.4 years), who were either cyclists or tri-athletes, for a period of 19 months.

As yet another alternative, some researchers have concluded that cortisol levels decrease with overtraining. For example, Lehmann et al (1992), found that resting cortisol levels decreased in a group of overtrained male distance runners (n = 8, mean age 33 years) after the training volume was increased by more than 100% (from 85.9 Km/week to 176.6

Km/week) over a 3-week period. Therefore, it is clear that the results concerning the behaviour of cortisol are discrepant in relation to overtraining.

Based on these discrepant findings, researchers have suggested that an increase in resting cortisol levels may be representative of sympathetic overtraining, while decreased resting cortisol levels may demonstrate parasympathetic overtraining. In general, the sympathetic form of overtraining is believed to represent an early transitory stage that shifts to the parasympathetic type after an individually varied amount of time (Urhausen & Kindermann, 2002). Table 2.8 suggests a likely time course of cortisol changes that occur with endurance athletes, based on when they transition from overreaching to overtraining syndrome. The review of literature indicates that cortisol concentrations change as overreaching advances to overtraining and then to overtraining syndrome. Table 2.8 illustrates the dynamic nature of the cortisol response, and may explain, in part, why physiologists disagree on the course of cortisol changes with overtraining (Armstrong & Van Heest, 2002).

Table 2.8. Cortisol changes during the transition from over-reaching to overtraining syndrome

HORMONE	TRAINING PHASE		
	Overreaching (or initial week of Overtraining)	Overtraining (sympathetic)	Overtraining Syndrome (parasympathetic)
Basal Cortisol at Rest	↔ or ↑	↔ or ↑	↓

↓ indicates decrease; ↑indicates increase; ↔ indicates no change

Adapted from Armstrong & Van Heest (2002)

E) *PHYSIOLOGICAL MECHANISMS AND METABOLIC EFFECTS OF INCREASED CORTISOL LEVELS:*

From the above discussion, it is clear that there is great variability in the cortisol response to overtraining. For the purpose of this review, the mechanism and the metabolic effects of an *increase* in cortisol is examined. This stems from the fact that a significant increase in cortisol likely signifies sympathetic overtraining or short term overtraining which is more prevalent in athletes than full-blown parasympathetic overtraining.

The exact mechanism responsible for an increase in cortisol levels is complex and controversial. One explanation for an increase in cortisol could be a decrease in pituitary sensitivity to cortisol negative feedback (Bonifazi et al, 2000). Other researchers suggest that this is not the case, but rather that a hypothalamic-pituitary dysfunction is involved (Fry et al, 1991b). Therefore, more research is needed in this area to determine the exact role of the H-P-A axis in relation to overtraining.

The metabolic actions of cortisol also need to be taken into account to explain the elevated concentrations of this hormone. Some of the effects of an increase in cortisol appear to be advantageous, however, the majority of metabolic effects appear to be detrimental with prolonged duration.

1. The Advantages to Hypercortisolism:

The advantages are generally brief in duration. The study by Luger et al. (1987), suggests that the immunosuppressive, catabolic, behavioural, and antireproductive effects of a hyperactive H-P-A axis may be beneficial in the short term. Specifically, a primary benefit to hypercortisolism is that it causes a change in fuel metabolism. High levels of

cortisol result in a significant increase in gluconeogenesis, which further leads to a substantial increase in glucose to be used as a fuel for exercise (Hadley, 1996). As well, the increase in cortisol leads to increased lipolysis and proteolysis. The free fatty acids and amino acids released can then be used as substrates for gluconeogenesis (Hadley). Also, an increase in cortisol leads to an anti-inflammatory effect (Hadley), which may allow an individual to exercise longer with a reduction in muscular soreness. Another advantage is that the increase in cortisol causes an increase in the synthesis of the catecholamines, epinephrine and norepinephrine (Hadley).

2. <u>The Disadvantages to Hypercortisolism:</u>

The disadvantages become apparent over time. According to Luger et al. (1987), the effects of hypercortisolism may be detrimental when they persist over prolonged periods. For instance, an individual with chronically high levels of cortisol runs the risk of a less than effective immune response and poor healing. Cortisol causes a decrease in the circulating levels of lymphocytes, which can result in failure of the body to provide antibodies necessary to fight infection (Hadley, 1996). Chronically high levels of cortisol can also lead to reproductive dysfunction. This is caused by high levels of CRH suppressing the secretion of luteinizing hormone-releasing hormone, and high levels of cortisol suppressing the hypothalamic-pituitary-gonadal axis at all levels, including the sex-steroid target tissues (Luger et al). Another disadvantage is that high levels of cortisol cause muscle wasting and weakness. The actions of cortisol on skeletal muscle are catabolic, resulting in proteolysis of muscle proteins (Hadley). Finally, chronically high levels of cortisol leads to elevated blood glucose levels which predisposes an individual to diabetes mellitus, by working against

the actions of insulin (Hadley). Therefore, hypercortisolism has certain short term advantages, but appears to be mainly detrimental with prolonged duration.

F) CONCLUSION:

To conclude, scientific studies demonstrate a relationship between exercise and cortisol. With regards to the acute effects of exercise, cortisol levels have been shown to increase proportionally in relation to increased duration and intensity of exercise. As well, the physical conditioning of an individual plays a role in cortisol levels.

Further, research has shown that resting cortisol concentrations can reflect long-term training stresses. In general, it appears as though overload training, which refers to a progressive increase in training stimuli, is associated with a significantly elevated cortisol level. It has been suggested that this increase in cortisol is a normal response to a dramatically increased training load. However, the increase in cortisol levels appears to be fairly temporary. In optimally trained athletes, tapering or a decrease in training load has been shown to decrease cortisol levels. Therefore, researchers have suggested that a decrease in cortisol levels is necessary for optimal performance.

On the other hand, athletes who are overtrained also exhibit alterations to their neuroendocrine system. Research regarding cortisol levels and overtraining is conflicting as researchers have variously suggested that cortisol levels increase, do not change, or decrease with overtraining. To make sense of this discrepancy, researchers have suggested that cortisol levels change over a specific time course when athletes transition from overreaching to overtraining syndrome. Specifically, it has been speculated that with overreaching there is either an increase or no change in resting cortisol levels. As

overreaching progresses to sympathetic overtraining (or short term overtraining) either an increase or no change in cortisol levels should again be observed. However, with parasympathetic overtraining (full-blown overtraining), it is believed that a decrease in cortisol levels will be exhibited. More overtraining studies need to be conducted in order to make definite conclusions regarding the cortisol changes that occur with overtraining.

Despite the need for further research, it appears that at the current time cortisol determination has the potential to be an effective marker to monitor the stress of training. Therefore, analyzing cortisol levels in athletes should help to evaluate training responses in order to avoid overtraining.

HEART RATE VARIABILITY AND ORTHOSTATIC RESPONSE: TOOLS FOR ASSESSING FATIGUE IN ATHLETES

Although the majority of research on fatigue and overtraining in athletes has examined hematological, immunological or hormonal markers, recently the use of heart rate variability has gained increasing popularity by researchers in the field of exercise science. Specifically, heart rate variability is a term that is used to describe both the oscillation in the interval between consecutive heart beats as well as the oscillations between consecutive instantaneous heart rates (Task Force of the European Society of Cardiology, 1996). The examination of heart rate variability is a non-invasive technique that is used to assess autonomic nervous system influences on the heart (James et al, 2002). This is of significant importance to athletes, as a disturbance of the autonomic nervous system has been suggested to account for some of the symptoms of overtraining and fatigue (Hedelin et al, 2000b).

The variations in heart rate may be evaluated by a number of methods. A very practical, and simple means of monitoring heart rate variability is the *dynamic postural heart rate test* (also known as the *Rusko* heart rate test) (Norris, 2001, unpublished). This method assesses the cardiovascular response to orthostatic stress, or assuming an upright stationary posture. Another more complex tool that can be used to determine heart rate variability is *spectral analysis*. This form of heart rate variability evaluation is based on a continuous electrocardiographic (ECG) recording.

This review describes the theoretical basis for these two forms of heart rate variability evaluation, and assesses the use of heart rate variability as a means of detecting a state of fatigue that could signify overtraining in athletes.

A) THEORETICAL BASIS OF HEART RATE VARIABILITY EVALUATION:

In its simplest form, heart rate and rhythm are largely under the control of the autonomic nervous system. The parasympathetic influence on heart rate is mediated by the release of acetylcholine by the vagus nerve, whereas the sympathetic influence on heart rate is controlled by release of epinephrine and norepinephrine. When a healthy individual is at rest, the variations in heart period are largely dependent on vagal modulation. However, there is constant interaction between the parasympathetic and sympathetic nervous systems (Task Force of the European Society of Cardiology, 1996). It is this premise, that heart rate variability is primarily under the control of the autonomic nervous system, that provides the basis for the theory behind both the dynamic postural heart rate test (orthostatic response) and spectral analysis. The specific mechanisms behind each of these methods are discussed further in the next section. The theoretical basis for the dynamic

postural heart rate test is examined first, followed by the theoretical basis for spectral analysis.

1. Theoretical Basis for the Dynamic Postural Heart Rate Test (Orthostatic Response)

As previously mentioned, the dynamic postural heart rate test requires an individual to shift from a supine to a standing position. The specific protocol for the test involves lying quietly for eight minutes and then standing up for two minutes. Heart rate measurements are taken every 5 seconds during the 10 minute test with the use of heart rate monitors (Norris et al, 2001, unpublished). The purpose of this test is to examine the initial heart rate response to the posture change, which is primarily mediated by the autonomic nervous system. Orthostasis, or assuming an upright position, causes a rapid reduction in cardiac output of approximately 10-15% (Savard & Stonehouse, 1995). This is normally compensated for by tachycardia. Researchers have shown that this increase in heart rate follows a specific time course, and that a dual mechanism is responsible for this adjustment (Neto et al, 1980; Borst et al, 1982). The primary mechanism for the immediate increase in heart rate has been termed the exercise reflex and results in a peak in heart rate about 3 seconds after standing briskly (Borst et al). The muscular effort of standing activates the heart rate increase by means of parasympathetic withdrawal and enhanced sympathetic outflow to the heart (Borst et al). Uusitalo et al (2000), also suggest that the body adjusts to the new standing position by increasing sympathetic activity and by influencing the distribution of blood volume. The secondary mechanism that is responsible for heart rate changes with standing is the arterial baroreceptor reflex. After approximately 5 seconds, the fall in arterial pressure corresponds with unloading of the

systemic baroreceptors. However, between 12 and 20 seconds, a rapid decline in heart rate occurs which is most likely due to reloading of the arterial baroreceptors (Borst et al). The baroreflex control of heart rate is predominantly mediated through vagal modulation and is essential in the regulation of blood pressure and heart rate changes (Uusitalo, 1998; Borst et al). However, sustained tachycardia during the later phases of standing would indicate that sympathetic stimulation had been maintained (Neto et al). Therefore, it is clear that the autonomic nervous system plays a major role in the heart rate response to posture change. The next section examines how spectral heart rate analysis detects changes in autonomic function.

2. Theoretical Basis for Spectral Analysis:

Spectral analysis is based on a standard electrocardiogram and has frequently been used to assess the cardiovascular responses to exercise (Seals & Chase, 1989). Measuring heart rate variability by means of power spectral analysis has many benefits. Power spectral analysis has the ability to identify frequency specific oscillations in heart rate signals that can be related to distinct physiological mechanisms and thereby provides an estimation of neurocardiac regulation (Taylor et al, 2003). Specifically, examining the fast (high frequency) and slow (low frequency) modulators of heart rate detects alterations in autonomic function on the heart (Hedelin et al, 2000b). The high and low frequency components can be quantified by means of power spectral analysis (Hedelin et al). High frequency (0.15-0.45 Hz) modulation of heart rate reflects vagal activity. Low frequency (0.04-0.15 Hz) modulation reflects both vagal and sympathetic activity (Hedelin et al; Sato et al, 1995). According to Hedelin et al, the low frequency component can be attributed to

fluctuations in blood pressure caused by sympathetic modulation, which result in parallel, vagally mediated changes in heart rate feedback through the baroreflex. Therefore, the signs of an adaptation in sympathetic activity on its own are difficult to interpret. With the problems associated with interpreting the low frequency component, many researchers have chosen to examine the low frequency/high frequency ratio. This ratio is a useful parameter that reflects the balance of autonomic nervous system activities (Sato et al).

B) *USE OF HEART RATE VARIABILITY WITH ATHLETES:*

Based on an understanding of the theoretical aspects of both the dynamic postural heart rate test and spectral analysis, it is clear that heart rate variability evaluation can provide useful information regarding the autonomic nervous system. The information obtained through heart rate variability is useful for researchers and clinicians to assess the cardiac autonomic function in diseased as well as healthy individuals (Taylor et al, 2003). Recently, the use of heart rate variability has gained increasing popularity in the field of exercise (Pichot et al, 2002). The research performed in this area may be critical to elite athletes. Several researchers have suggested that heart rate variability analysis is a useful tool for monitoring the effects of physical training loads on performance and fitness. As well, it has been suggested that the use of heart rate variability could eventually be used to detect and prevent overtraining states in athletes (Pichot et al). The following sections of this review examine the influence of physical training on heart rate variability with specific emphasis on endurance training. As well, the effects of overtraining on heart rate variability are examined.

1. Influence of Endurance Training on Heart Rate Variability:

Endurance training has been postulated to result in an adaptation of the autonomic nervous system reflected by changes in several cardiovascular variables at rest as well as in alterations in the reflex control of the circulation (Seals & Chase, 1989; Hedelin et al, 2001). For instance, endurance trained athletes typically have a low resting heart rate (Loimaala et al, 2000; Pichot et al, 2002; Janssen et al , 1993). Research has suggested that training induced resting bradycardia results from increased cardiac parasympathetic modulation, and decreased cardiac sympathetic modulation (Uusitalo, Uusitalo et al, 1998).

In addition, heart rate variability has been suggested to change with endurance training. Table 2.9 provides a summary of studies in which the authors examined the effects of moderate endurance training on heart rate variability.

As can be seen from Table 2.9, the majority of research in this area has concluded that endurance training with positive training effects (enhanced performance) induces an increase in parasympathetic tone in relation to sympathetic tone, which can be detected by increased heart rate variability (Uusitalo et al, 2000; Seals & Chase, 1989; Iellamo et al, 2002). For example, the study by Seals & Chase, which examined 11 male subjects (aged 53 ± 2 years) before and after 30 ± 1 weeks of moderate endurance training, found that the endurance training led to increased heart rate variability, signifying increased parasympathetic modulation. Similarly, Portier et al (2001), determined that a 12 week endurance training program resulted in increased parasympathetic modulation, and an increase in the high frequency component of heart rate variability in 9 elite marathon runners (6 males and 3 females; aged 29-35 years).

Table 2.9. Effects of Moderate Endurance Training on Heart Rate Variability

REFERENCE	STUDY DESIGN	FINDINGS
Seals & Chase (1989)	11 male subjects (ages 53 ± 2 years) studied before and after 30 ± 1 week of endurance training (plus 8 controls)	Endurance training lead to ↑ HRV; ↓ HR at rest; baroreflex control of HR unchanged
Loimaala et al (2000)	83 sedentary men → 28 controls (aged 47.0±5.0 years), 27 low intensity ex. Program (aged 45.6±6.2 years), 28 high intensity ex. Program (aged 46.8±5.6 years) → 5 months	No significant changes in HRV or BRS in either exercise group.
Iellamo (2002)	7 elite male rowers (all aged 18 years) studied for 9 months (↑ training loads up to 75% and 100% of max)	Training load at 75% maximum: ↑ vagal and ↓ sympathetic modulation
Hedelin, Bjerle (2001)	17 x-country skiers (9F, 8M) & 7 canoeists (2F, 5M) (mean age 18.5±1.8 years) studied before & after 7 month training period	- HF & total HRV did not change with changes in muscle performance / aerobic capacity - Improved VO_2 max dependent on ↑ HF (reflects parasympathetic activity)
Pichot et al (2002)	6 sedentary male subjects (aged 32.7±5.0 years) → 2 months endurance training → 1 month overload → 2 weeks recovery	2 months of endurance training led to predominance of parasympathetic modulation
Portier et al (2001)	9 elite marathon runners (6M, 3F; aged 29-35 years) tested after 3 weeks rest & after 12 week endurance training program	-Intense endurance training led to ↑ HF (↑ parasympathetic modulation) -lowered orthostatic tolerance - ↓ in sympathetic mod. during supine and tilt positions
Pichot et al (2000)	7 male middle distance runners (mean age 24.6±4.8 years) → 3 weeks heavy endurance training → 1 week rest	During rest week: ↑ parasympathetic and ↓ sympathetic modulation

HF = high frequency, HRV = heart rate variability, HR = heart rate, BRS = baroreflex sensitivity

There is also consistent evidence indicating that enhanced parasympathetic activity is associated with increased maximal oxygen uptake (VO_2 max), which is a common measure of fitness level (Hedelin et al, 2001; Pichot et al, 2002; Iellamo et al, 2002). For instance, Hedelin et al, found that improved VO_2 max was dependent on an increase in the high frequency component of heart rate variability, which reflects parasympathetic activity. This study looked at 17 cross country skiers and 7 canoeists before and after a 7-month endurance training program.

Despite these findings, there has been some controversy regarding the adaptations of the autonomic nervous system with endurance training. Some researchers have found no significant changes in heart rate variability with endurance training. Loimaala et al (2000), examined 83 sedentary men, of whom 28 (aged 47.0±5.0 years) were controls, 27 (aged 45.6±6.2 years) took part in a 5 month low-intensity exercise program, and 28 (aged 46.8±5.6 years) took part in a 5 month high-intensity exercise program. The results of this study suggested that neither of the exercise groups experienced changes in heart rate variability or baroreflex sensitivity.

These inconsistencies in the literature might be attributed to several factors. Firstly, differences in the methods of determining heart rate variability and baroreflex sensitivity may have contributed to the differences. Secondly, inter-individual variability or non-training related differences between subjects may have caused the inconsistencies (Iellamo et al, 2002). Thirdly, the athletic backgrounds and the fitness level of the subjects may have substantially affected the results. For example, Iellamo et al suggested that highly trained athletes may require adaptational changes in the neural control of circulation that could be different from those brought about by moderate intensity training

in non-competitive athletes and sedentary individuals. Lastly, the volume and intensity of the training programs that the subjects underwent in the studies may have had a large impact on the heart rate variability measures. Research has shown that normal endurance training may cause different changes in heart rate variability compared to excessive training (which may involve significant increases in training volume and/or intensity) (Uusitalo, 1998). The effects of excessive training leading to fatigue or short term overtraining is examined in the next section in relation to heart rate variability.

2. Effects of Overload Training / Short Term Overtraining on Heart Rate Variability

Most endurance athletes undergo periods of excessive or very intense training in order to induce a state of fatigue (overload training), which is followed by an increase in physical capacity after a recovery period (supercompensation). However, the accumulation of too much fatigue or lack of an appropriate recovery period can result in overtraining, which can seriously compromise a competitive season. Overtraining induces an imbalance in the autonomic nervous system, characterized by a predominance of either the parasympathetic or sympathetic nervous system, depending on the type of overtraining (Pichot et al, 2002). The sympathetic form of overtraining is believed to cause an increase in resting heart rate, blood pressure and sympathetic neuroendocrine activity. The parasympathetic form is believed to be characterized by a fall in resting heart rate and blood pressure, and an increase in parasympathetic activity (Portier et al, 2001). A mixture of the two forms can exist, because the sympathetic type represents an early transitory stage that shifts to the parasympathetic type after an individually varied amount of time

(Urhausen et al, 1995). Researchers have suggested that the parasympathetic form of overtraining reflects exhaustion of the autonomic nervous system in general (Portier et al).

Analysis of the variability in beat to beat changes in heart rate, observed through spectral analysis, has been proposed as a tool to help in the diagnosis of overtraining (Urhausen & Kindermann, 2002). Researchers have speculated that the sympathetic and parasympathetic forms of overtraining are reflected by corresponding changes in the low and high frequency components of the spectral analysis (Urhausen & Kindermann). Table 2.10 summarizes the results of studies that have examined the effects of overload training or overtraining on heart rate variability.

Based on the majority of the findings summarized in Table 2.10, it appears as though a decrease in heart rate variability, signified by an increase in sympathetic modulation, is a sign of impending fatigue. Specifically, the literature suggests that very intensive endurance training shifts the cardiovascular autonomic modulation from a parasympathetic toward a sympathetic predominance (Iellamo et al, 2002). For example, Pichot et al (2002), who examined 6 sedentary subjects, found that during 2 months of endurance training there was a predominance of parasympathetic modulation, however during the following month of overload training, there was a stagnation of parasympathetic activity associated with an increase in sympathetic activity. As well, following the overload training, there was a week of recovery in which there was a significant rebound in parasympathetic activity. The results of this study were similar to another study by Pichot et al (2000) which observed 7 middle distance runners. This study concluded that 3 weeks of heavy endurance training (overload training) led to decreased parasympathetic modulation and increased sympathetic modulation. The subjects in both of these studies were not diagnosed with

overtraining, but they did experience overload training. Therefore, based on these results, it appears as though heart rate variability analysis is an appropriate tool to monitor the effects of varying training loads as well as cumulated physical fatigue (Pichot et al, 2002; Pichot et al, 2000).

Table 2.10. Effects of overload training on heart rate variability

REFERENCE	STUDY DESIGN	FINDINGS
Pichot et al (2002)	6 sedentary male subjects (aged 32.7±5.0 years) → 2 months endurance training → 1 month overload → 2 weeks recovery	-endurance training → predominance of parasympathetic -overload → ↑ in sympathetic -recovery → rebound in parasympathetic
Uusitalo (1998) * Uusitalo et al (2000) * Uusitalo, Uusitalo, Rusko (1998)*	9 female athletes (aged 23.9±3.0 years) → 6-9 weeks of heavy endurance training → 5 diagnosed as overtrained → plus 6 controls	-heavy endurance training & OT did not change BRS but LF ↑ (↑ sympathetic modulation) -heavy training / OT induced a ↓ in max aerobic power → related to ↓HRV during standing (↑ sympathetic modulation) - resting HR ↓ with OT /heavy endurance training - ↑ VO_2 max correlated with ↑ parasympathetic modulation
Hedelin, Kentta (2000)	9 elite canoeists (6M, 3F; aged 18-23 years) studied for a 6 day intense training camp	-no changes in HF or LF HRV at rest or following tilt with short-term intensive endurance training.
Pichot et al (2000)	7 male middle distance runners (aged 24.6±4.8 years)→ 3 weeks heavy endurance training → 1 week rest	-overload training led to ↓ parasympathetic and ↑ sympathetic modulation -suggest HRV good indicator of cumulated training load
Hedelin, Wiklund (2000)	One male junior x-country skier (age 16 years) diagnosed with overtraining syndrome	- Extensive parasympathetic modulation with OT (↑HRV particularly in HF range) - ↓ resting HR
Iellamo et al (2002)	7 male elite rowers (all aged 18 years) studied for 9 months (↑ training loads up to 75% and 100% of max)	- very intensive endurance training (100% max) → shift from parasympathetic toward sympathetic predominance - suggest ↑ sympathetic adaptation for ↑ athletic performance

* all 3 articles relate to the same study
LF=low frequency, HF= high frequency, HRV=heart rate variability, BRS=baroreflex sensitivity, OT=overtraining

Other researchers have also found heart rate variability to be an effective tool for diagnosing overtraining. Uusitalo et al (1998, 1998 and 2000) wrote three articles based on the results obtained from 9 elite athletes who underwent 6-9 weeks of heavy endurance training. Of these 9 female athletes, 5 were diagnosed with overtraining. Based on results of these 5 athletes, it was concluded that overtraining was related to an increase in cardiac sympathetic modulation. Therefore, research suggests that heart rate variability was useful in detecting the cardiac autonomic adaptations that occurred with sympathetic overtraining.

3. Long Term Overtraining: Effects on Heart Rate Variability:

As previously mentioned, it has also been suggested that beat-to-beat changes in heart rate may also be beneficial in detecting parasympathetic overtraining. Hedelin et al (2000b) examined the effects of overtraining syndrome on heart rate variability. This case study investigated the cardiac autonomic adaptations of a cross-country skier who presented with reduced performance in competitions, early breathlessness during training and extreme fatigue. The athlete was advised to rest and required a rest period of 2 months in order to regain previous work capacity. When this athlete was examined, it was found that there was a shift towards increased heart rate variability, and a reduced resting heart rate. These findings suggested a cardiac autonomic imbalance with extensive parasympathetic modulation in this athlete when overtrained. Despite the limitations of a one-subject study, the results of this study provide support for the theory of parasympathetic overtraining.

4. Use of Heart Rate Variability for Preventing Overtraining:

Based on the previous discussion, it appears as though heart rate variability analysis is an appropriate tool for diagnosing training loads and cumulated fatigue. As well, analysis of the autonomic nervous system appears to be a critical parameter in over-reaching and overtraining. Despite the fact that more research is needed to determine if heart rate variability can diagnose overtraining, it is likely that the information obtained can be used to prevent overtraining. According to Pichot et al (2002), heart rate variability analysis could be helpful in finding target values to optimize over-reaching and to determine threshold values beyond which there would be a risk of overtraining. Moreover, examining an athlete's heart rate variability assessment would enable a coach to judge how well the athlete's cardiovascular system is adapting to training or competitions. This analysis would aid the coach in subsequent training, allowing for adjustments in the volume and intensity of training based on the athlete's actual functional state (Omegawave Operating Manual, 2002). Therefore, it is clear that the use of heart rate variability is helpful in managing the training process and in preventing overtraining.

C) CONCLUSION:

In conclusion, this review of literature has shown that heart rate variability is a practical and reliable marker of fatigue. Heart rate variability is most commonly assessed through power spectral analysis, which is based on a continuous electrocardiographic (ECG) recording. However a more simplistic dynamic postural heart rate test also provides valuable information on the beat-to-beat changes in heart rate. Both of these tests can provide useful data regarding the autonomic nervous system. This is especially important

for athletes in order to detect and prevent overtraining states. Based on a close examination of the literature in this area, it is well supported that regular endurance training enhances parasympathetic modulation. As well, research has suggested that typical endurance training causes different changes in heart rate variability compared to excessive endurance training. Several studies have confirmed that very intensive endurance training shifts the cardiovascular autonomic modulation from a parasympathetic toward a sympathetic predominance. These findings suggest that decreased heart rate variability, or increased sympathetic activity is a sign of impending fatigue. If the athlete is provided with sufficient recovery, a rebound in parasympathetic activity has been observed. However, with insufficient recovery sympathetic overtraining may occur. Subsequently, if sympathetic overtraining shifts to parasympathetic overtraining, a cardiac autonomic imbalance with extensive parasympathetic modulation may be observed. However, these suggestions need to be confirmed with more subjects, and the inconsistencies in the literature need to be evaluated further. In the meantime, it appears as though heart rate variability evaluation is a very useful tool for evaluating training loads and for identifying a state of fatigue.

PERFORMANCE MEASURES

A decrease in performance despite continued training is the most indicative marker of overtraining. Decrements in competitive performance of 1% -20% have been reported in overtrained athletes (Mackinnon, 2000). In elite sport, even a small decrement in performance may be detrimental to the athlete (especially for athletes who may have invested years into preparing for a single competition, such as the Olympics). According to

Mackinnon, in competitive swimming, only a 4% -5% difference separates times required to qualify to compete in the Olympics from World record times. For the purpose of this study, focus is placed on competitive swimmers, and the measures that are appropriate to evaluate performance in this sport.

A) *SPORT SPECIFIC TESTS:*

Performance incompetence can be specifically tested using sports-specific performance tests (Steinacker et al, 1999). For competitive swimmers, two tests that have been identified as predictors of performance include the 5 x 200m set and the 4 x 50m set (Norris et al, 2001, unpublished). The two tests outlined are situated in a pool setting, for reasons of specificity and practicality (Pyne et al, 2000b). A description, along with the rationale for these two tests, follows.

1. 5 x 200m:

The 5 x 200m heart rate step test is a measurement tool designed to provide information on the aerobic fitness of the swimmer. The standard protocol is to swim a graded incremental test through the 5 swims. Each 200m is swum using freestyle or backstroke on a 4 minute interval, for a total test time of 20 minutes. Each 200m is swum progressively faster, such that the athlete's heart rate should progress from approximately 60 beats below maximal heart rate for the first swim to 20 beats below maximal heart rate for the last swim. The swimmers are instructed to start each 200m from a push in the water, and to even split each 200m swim (i.e. swim the first 100m at the same speed as the second 100m). Heart rate measurements are taken immediately at the end of each 200m,

to determine cardiovascular responses to increased swimming speeds (Pyne et al, 2000b). The data obtained is used to create a heart-rate velocity curve. If fitness has deteriorated, then the curve will shift upward or leftward. As well, a higher heart rate than usual during the test may be indicative of illness, or an inability to adapt to the current training load (Pyne et al). According to a review article by Hopper & Mackinnon (1995), heart rate after standardized submaximal exercise tests is a practical tool for monitoring training loads. As well, this test can be used to predict the athlete's 400m swim time. This is done by extrapolation of the slope of the heart-rate velocity graph to maximal heart rate. The speed (meters/second) that the swimmer should be able to achieve at maximal heart rate is used to calculate the 400m time. Therefore, the test can be used as a performance predictor as well as an indicator of fatigue.

2. 4 X 50m

The 4 x 50m test is used to determine the anaerobic power of the swimmer. The protocol for the test involves swimming 4 x 50's of the athlete's main stroke on a 2 minute interval. The swimmers are instructed to hold the best average that they can for the 4 swims. This means that they should be swimming at maximal effort for each interval. This set is used to provide a prediction of the athlete's 100m speed. This prediction is done by simply doubling the average of the 50m's swum (Norris, 2001, unpublished).

B) TIME TRIALS

Time trials are also useful indicators of swimming performance. Time trials may be of benefit when competitions are not occurring. According to Hooper et al (1999), time

trials should be used as performance measures because swimmers do not compete at swim meets on a regular basis. As well, elite swimmers should be familiar with time trials, and therefore maximal effort should be expected (Hooper et al). Therefore, time trials may be used frequently to evaluate improvements or decrements in performance. For this study, a 100m maximal swim was performed by the swimmers which was used to assess their anaerobic capabilities during the season.

C) SWIM COMPETITIONS

A decrement in performance, with an increasing feeling of fatigue, is the main sign of overtraining (Uusitalo, 2001). According to Fry et al (1991a), results obtained from athletic competitions are the most valid index of sporting form, since they are the only true expression of an athlete's readiness to perform. However, competitions provide little information regarding the contributing factors to performance, or why the performance was poor (Fry et al). This suggests that if competition performance is not up to the athlete's potential, then further laboratory tests could be conducted to determine why performance was not optimal (Fry et al).

PART IV - METHODOLOGICAL CONSIDERATIONS

The preceding section examined tools for monitoring training, which included a variety of hematological, immunological, hormonal, cardiovascular and performance measures. As is likely clear from the discussion of the aforementioned tools, there is still great debate by researchers in the area of exercise science as to what the best tools are for assessing overtraining in athletes. A probable reason that no single parameter is an

agreed-upon marker of overtraining is that there are frequent inconsistencies in the literature. Factors related to the inconsistencies include a lack of internationally standardized terminology, a relatively small number of overtrained athletes studied, and difficulties differentiating normal from abnormal training responses (Hopper & Mackinnon, 1995). As well, research discrepancies may be explained by methodological inconsistencies (Tremblay & Chu, 1994). In order to ensure valid experimental outcomes, researchers must exercise caution when designing and administering methods to minimize interference by confounding variables. The following section reviews sources of analytical and biological variation, as well as factors that should be considered and /or standardized when researching overtraining in athletes.

A) ANALYTICAL VARIATION:

The first source of variation to be discussed is analytical variation. This type of variation refers to the differences in the measurement of a sample after it has been prepared for analysis (Tremblay & Chu, 1994). There are a variety of sources that have the potential to cause inaccuracy or bias within an analytical procedure. Examples of analytical variation include assay method, instrumentation, reagents and standards, and the skill of the staff performing the test (Tremblay & Chu). The analytical variation is expressed as the standard deviation (SD) and/or the coefficient of variation (CV) of the assay. Acceptable coefficients of variation for most biological assays are 6% -15% (Chard, 1987). Unfortunately, many assays do not achieve such an analytically precise goal. Therefore, researchers need to select assays that minimize the assay imprecision. To best achieve this, both intra-assay (within-run) and inter-assay (between run) variation should be

controlled for. Intra-assay variation can be minimized by having the same researcher run all of the samples for one subject. Inter-assay variation can be minimized in repeated design studies (similar to this one) by batching all of the samples from the same individual and assaying them all together (Tremblay & Chu).

B) *BIOLOGICAL VARIATION*

The second source of variation to be discussed is biological variation. This type of variation refers to the differences in the true level of a substance within an individual over a period of time. Biological variation occurs as a result of differences in emotional state, stress, health, diet, exercise, environment, seasonal activity and genetic make-up (Tremblay & Chu, 1994).

Based on a knowledge of the various sources of variation, it is clear that there are a number of factors that need to be considered and/or standardized when studying athletes for the purpose of overtraining research. As a result, the factors that were considered when analyzing the variables in this study included age, gender, diet, previous exercise and the circadian rhythm of some of the variables.

1. Age:

When studying a group of athletes for overtraining, it is essential that age and maturation level are taken into account. Prepubertal individuals exhibit higher or lower levels of blood markers and hormones compared to adults (Tremblay & Chu, 1994). Therefore, it is ideal if subjects are fairly close in age and have already reached puberty. The athletes used in this study were between the ages of 16 -23 years and had all reached

puberty (i.e. all of the female subjects were menstruating and all of the male subjects had visible facial and body hair along with deep voices).

2. Gender:

Several gender differences exist between blood markers and various hormones, especially after puberty. For example, Tables 2.3 and 2.5 illustrate the differences between the reference ranges for males and females for the numerous red and white blood cell indices. However, due to statistical reasons, it was not feasible to separate the males and the females for many of the variables because of the small number of subjects.

Similarly, several gender differences in hormonal responses have been identified. Most notable are the differences found between the sex hormones. For example, testosterone levels are five times higher in men than in women. As well, women have specific hormonal responses that are associated with their menstrual cycle. Fortunately for this study, it has been suggested that cortisol secretion is not affected by menstrual cycle phase (Stewart et al, 1993). Therefore, it was not necessary to record the phase of menstruation in the female athletes studied at each testing time. However, the females in this study did keep track of their menstrual cycle in their training logbooks.

3. Diet:

The nutritional status of the subjects could alter the hormone concentrations. However, it was suggested in a review article by Urhausen et al (1995), that short-term dietary modifications (3 days) were not associated with changes in cortisol concentrations.

Other researchers have suggested that cortisol determination should be done when the individual is in a fasted state.

Similarly, there is evidence that food intake can influence plasma glutamine levels, especially if the meals contain a substantial protein content. In order to control for this, it has been suggested that an 8-hour fast is needed before sampling for plasma glutamine (Rowbottom et al, 1996). There are few current studies on glutamate, however it can be postulated that glutamate may react in the same way as glutamine due to the fact that glutamate is the precursor to glutamine.

Therefore, taking these variables into consideration, the subjects in this study were asked to fast for 8 hours prior to coming to testing in the morning. However, it was unrealistic to have the subjects in a fasted state when giving their evening saliva samples, which were used for cortisol determination.

4. Previous Exercise:

Previous exercise sessions may affect hormonal results. Fry, Morton and Webb (1991), examined the hormonal response to intense interval training, and found that recovery values of the hormones were depressed even 24 hours later. This suggests that it is necessary to perform the hormonal measurements after a day of rest for the athletes.

It is also probable that previous exercise may have an effect on some of the other variables measured. For instance, a study by Gleeson et al (1995), suggested that saliva samples to be used for S-IgA determination should be collected a minimum of 24 hours after the previous training session to reduce the physiological variability of S-IgA due to

flow rate, dehydration and prior exercise effects. Therefore, all of the main test days in this study were done after a day of rest for the subjects.

5. Circadian Rhythm:

Several of the variables examined in this study exhibit diurnal variation. This refers to the fact that their concentrations fluctuate throughout the day. Specifically, the variables that need to be controlled for time of day of testing include cortisol, glutamine, glutamate and S-IgA.

Cortisol exhibits profound diurnal variation. Evening cortisol values have been recorded to be about half of the morning values. Specifically, cortisol concentration is at its lowest near midnight, with its highest values occurring between 8 AM and 10 AM. The reason for diurnal variation may be related to activity level, stress, wakefulness and sleep, food ingestion, and daylight and darkness (Tremblay & Chu, 1994).

Similarly, glutamine levels fluctuate throughout the day. This diurnal variation may be linked to cortisol secretion, as the physiological levels of cortisol have profound effects on the rate of release of muscle glutamine (Rowbottom et al, 1996).

Further, S-IgA also exhibits diurnal variation. According to Walsh et al (2002), S-IgA levels peak at approximately 8:00 AM and fall significantly throughout the morning to a plateau at 12:00 PM.

Therefore, researchers must be aware of this inherent diurnal variation among these variables. To help control for this variation, repeated testing should always be done at the same time of day. With respect to this study, efforts were made to ensure that the

subjects provided their saliva or blood samples at the same time of day for each of the different test days.

C) *SUMMARY OF METHODOLOGICAL CONSIDERATIONS:*

Based on the above discussion, there are a number of factors that need to controlled or standardized when monitoring athletes for overtraining. A summary of these factors is listed in Table 2.11.

Table 2.11. Factors that Should be Controlled When Measuring Physiological Markers.

STANDARDIZED CONDITIONS:
Time of day
Testing environment: humidity, temperature, light
Use of caffeine, alcohol, tobacco
Nutrition and previous meal
Medications
Health status
Menstrual cycle
Training history
Training volume and intensity during previous days
Time interval to previous exercise
Quality and quantity of sleep
Stress level
METHODOLOGICAL FACTORS:
Posture
Identical collection, transportation, storage and analysis protocols

Adapted from Uusitalo (2001)

CHAPTER THREE: METHODOLOGY

OVERVIEW:

Fatigue and under-performance were examined in 14 elite competitive swimmers in this 10-week prospective study. The variables that were investigated include hematological measures (CBC and iron profile), immunological measures (S-IgA, glutamine and glutamate, NK cells), hormonal measures (cortisol), cardiovascular measures (Rusko heart rate test and spectral heart rate variability), as well as performance measures (test sets, time trials and swim competitions). For this study, the 14 swimmers served as their own controls and therefore testing was done twice before their competitive season started in order to establish baseline values for each subject. Three other testing days were conducted at different phases of the swimmers' training macrocycle. See Figure 3.1 for a schematic overview of the study. Specifically, testing was conducted five times during the 3 month period as follows:

1.) Pre Season 1: one week prior to the commencement of the season in order to establish **baseline** values for each subject (September 9th).

2.) Pre Season 2: three days prior to the start of training to validate the **baseline** data collected (September 12th).

3.) Mid-season 1: four weeks into training (October 15th) to determine normal training responses. The tests were conducted at the end of a **build** microcycle.

4.) Mid-season 2: eight weeks after the season commenced (November 12th) to determine training responses to overload training. Testing was performed at the end of a **crash** microcycle.

5.) Prior to Major Competition: testing was performed 3 days prior to the subjects' major competition for the short course season, at the start of the second week of taper (November 25th). The swimmers competed in either the Canadian National Swimming Championship that was held in Edmonton, or the Prairie Winter Invitational that was held in Winnipeg. Both competitions ran from November 28th until December 1st, 2002. During this time period the athletes should have been at their optimal level of readiness to perform.

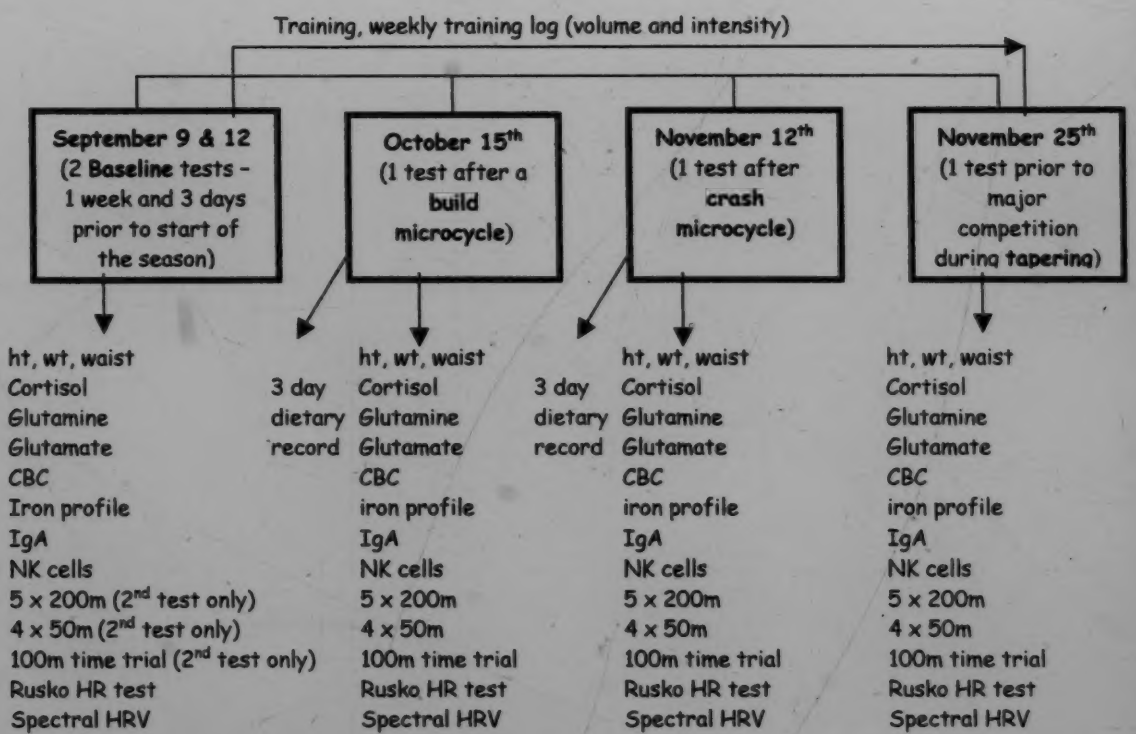

Figure 3.1. Schematic Overview of the Study.

ETHICAL CONSIDERATIONS:

For this study, the experimental procedures were reviewed and approved by the Faculty of Physical Education and Recreation Ethics Committee in August, 2002. Before

participating in the study, the subjects were briefed on all testing procedures during an orientation meeting that was held at the Kinsmen Sports Center at the end of August. Subjects were also provided with a study information package that outlined the purpose of the study, the requirements of participation, the time commitment involved as well as the associated risks and benefits of participation (Appendix B). The subjects were given time to review the participant information prior to signing an informed consent sheet (Appendix C). Subjects under the age of 18 years also obtained parental or guardian consent before participating in the study (Appendix D).

The risks associated with participation were minimal. Subjects were informed that they may experience bruising and/or infection at the venipuncture collection site. However, having a registered nurse trained in taking blood samples helped to minimize this risk. As well, all efforts were made to ensure that universal precautions were followed and that the blood was taken in a sterile environment. No significant adverse effects associated with participation were reported.

SUBJECTS:

The subjects, aged 16 -23 years, recruited for this study were 14 elite competitive swimmers (8 female, 6 male) from the University of Alberta Swimming Center (UASC). These subjects volunteered to participate in the study prior to the orientation meeting that was held in August. Subjects were recruited from this club due to the fact that the UASC is the primary elite competitive swimming club within Edmonton, and also because the primary researcher was familiar with the team. Two of the subjects recruited were sprinters, 8 were middle distance swimmers and 4 were distance swimmers. It was only

feasible to study 14 swimmers due to the expense of the tests performed. Initially, there were 15 subjects participating in the study, but unfortunately one swimmer did not complete the final testing day and failed to turn in his training log book, one of his 3 day dietary records and numerous saliva samples. Therefore, this subject was eliminated from the study as a result of missing data.

Inclusion criterion for this study included previous qualification for the Canadian National Championships, and a minimum of 4 years of competitive swimming experience. Despite these inclusion criteria, 2 of the subjects unfortunately failed to qualify for the Canadian National Championships in November, 2002. Although these 2 swimmers had qualified in the past, they both missed the National standard in their respective best events. These two swimmers therefore attended the Prairie Winter Invitational swim meet that was held in Winnipeg the same weekend as the Canadian National Championships (November 28th to December 1st, 2002).

Swimmers would have been excluded from this study if they were smokers, if their parents refused participation (for subjects under 18 years of age), or if they were under the age of 16 years. No swimmers had to be refused participation for any of these reasons.

TRAINING:

The length of the training period studied was 10 weeks, commencing September 16th (pre-season) and going through until November 25th (during the middle of a taper). Each swimmer trained according to a program designed by his/her own coach. There were two coaches involved in the study. One of the coaches was primarily responsible for the sprint and middle distance swimmers, while the other coach trained the distance swimmers.

Therefore, each subject swam an individual amount of mileage and came to different practices based on his or her coach's instructions. To account for this, subjects were instructed to keep a daily training log (Appendix E). In the log book, the swimmers recorded the volume (distance) of each practice they attended, their perceived intensity and fatigue based on a 7 point scale for that practice, time spent doing dryland activities, as well as feelings of illness and menstrual cycle information (for the females). One of the coaches also provided an attendance record along with a daily volume log for each of the swimmers that he coached. This was helpful in validating the entries that the swimmers made in their personal logbooks.

TESTING SCHEDULE:

The testing involved invasive and non-invasive measures. The invasive measures obtained included CBC and differential, iron profile, cortisol, glutamine/glutamate, NK cells and S-IgA. The non-invasive measures included: a dynamic postural heart rate test (Rusko), spectral heart rate analysis, sport specific tests (5 x 200m, 4 x 50m), a time trial (100m maximal effort swim), and a major swim competition (either the Canadian National Swimming Championships or the Prairie Winter Invitational). As well, body weight, height, and waist circumference data were collected, body mass index (BMI) was calculated, and 3-day dietary records were analyzed in order to obtain subject characteristics. All of these variables were evaluated at various times throughout the three-month study period. Table 3.1 illustrates the testing schedule that was used for this study.

Table 3.1. Testing Schedule

Variable	Measuring Tool	Mon Sept 9	Thur Sept 12	Mon Oct 14	Tues Oct 15	Mon Nov 11	Tues Nov 12	Fri Nov 22	Sat Nov 23	Sun Nov 24	Mon Nov 25
Subject characteristics	Height	x	x		x		x				x
	Weight	x	x		x		x				x
	Waist	x	x		x		x				x
	BMI	x	x		x		x				x
	3-day diet record				Sun, Mon, Tues		Sun, Mon, Tues				
Hematological	RBC	x	x		x		x				x
	Iron profile	x	x		x		x				x
Immunological	WBC & differential	x	x		x		x				x
	Glutamine / glutamate	x	x		x		x				x
	% NK cells; % CD4; % CD8; CD4/CD8	x	x		x		x				
	Salivary IgA	x	x		x		x				x
Hormonal	Salivary Cortisol	x	x	x	x	x	x			x	x
Cardiovascular	Rusko HR test	x	x		x		x				x
	Spectral HRV	x	x		x		x				x
Performance	5 x 200m test set		x		x		x	x			
	4 x 50m test set		x		x		x		x		
	100m time trial		x		x		x		x		

Note: the major competition occurred Nov. 28th - Dec. 1st

Basically, during each of the five testing sessions, height, weight, and waist circumference were measured, and both of the cardiovascular tests were performed. As well, at each testing session for all of the 14 subjects, venous blood was collected and a

saliva sample was obtained in order to analyze the hematological, immunological and hormonal variables. Furthermore, each subject provided evening saliva samples that were analyzed for cortisol. On the first and second test days, the evening saliva samples were done that same evening, but for the 3rd, 4th and 5th test days, the evening saliva samples were performed the night preceding the main test day. The reason for this was that the evening cortisol samples needed to be taken after a day of inactivity (Tremblay & Chu, 1994). As for the performance measures, the 5 x 200m test set, the 4 x 50m test set and the 100m time trial were conducted at all of the testing sessions except for the first one. It was believed that due to the experience level of the swimmers, and their familiarity with the performance tests, these performance measures would not change significantly in 3 days and therefore one baseline value was sufficient. For the last test session however, the performance measures were spread out over 3 days. The reason for this was that the subjects and coaches were worried that their taper may have been compromised if all of the testing was done too close to the actual competition.

TEST DAY PROTOCOL:

A) PRECEDING EVENING:

Subjects were asked to give two salivary samples on the evening prior to the morning testing sessions. Salivary samples needed to be given between 4 and 5 PM and again between 8 and 10 PM. Subjects were asked to chew on a cotton swab and then expectorate the swab into a plastic tube, store the sample in their freezer overnight, and bring the sample with them to testing the following morning. These salivary samples were used for cortisol determination. As well, the subjects were asked to fast for 8 hours prior to coming

in for testing in the morning (including no caffeine or alcohol). Vitamins and medications were recorded for the 3 days prior to the testing (Hooper et al, 1993) (Appendix F).

B) *MORNING TESTING SESSION (Figures 3.2a and b):*

Formal testing was conducted in the morning between 6 and 9 AM five times during the course of the study. On the day of testing, the subjects arrived at the University of Alberta and immediately placed their saliva samples from the night before into a -20°C freezer. The swimmers came in two separate groups. One group arrived at 5:45AM and the second group arrived at 7AM. This helped to decrease the number of subjects at each testing station. Upon arrival, the subjects were directed to one of three stations. At station number one the subjects had their weight, height, and waist circumference taken by a trained anthropometrist. At station number two, the subjects performed both the spectral heart rate analysis (6 minute test) and the Rusko heart rate test (10 minute test). The third station required the subjects to give a saliva sample, followed by a blood sample from an antecubital vein taken by a registered nurse (3 x 5ml vacutainers were filled). The saliva sample was immediately frozen and stored at -20°C until analyzed for S-IgA (Krzywkowski, Petersen, Ostrowski et al, 2001) and cortisol. The blood samples were placed on ice until the morning testing session was completed. Once the blood sample was given, the subjects rested in a chair for approximately 10-15 minutes during which time a recovery-stress questionnaire (RESTQ-76 Sport) was completed. This questionnaire has not been previously described as the results will not be discussed in this study. An analysis of the questionnaire will be made at a later date. The questionnaire was scheduled after the blood sample so that the subjects could have time to relax after giving blood. The order of

these three stations were randomized in order to accommodate more subjects at the same time (i.e. two subjects started with station #1, two subjects started with station #2, and 2 subjects started with station #3. The subjects then switched stations once completed). The stations were randomized because it was believed that results from one test would not affect the next test (based on the fact that recovery time was given after the blood test before the cardiovascular tests were performed).

Following completion of all three stations, the subjects were sent to the pool for the swimming tests. The subjects were allowed to warm-up in the pool following a standardized 1000m workout (Appendix G). After warm-up, the swimmers completed the 4 x 50m swim test. Following that, a 400m warm-down was allowed. The last test done in the morning was the 100m time trial (maximal effort). The swimming time was used as a criterion marker of performance.

Immediately following completion of the morning testing session for all of the subjects, 2 vacutainers of each subject's blood were delivered in a biohazard container to the Dynacare Kasper Medical Laboratory at the University of Alberta Hospital for CBC and differential and iron profile determination. A sample of whole blood from each subject's third vacutainer was refrigerated for immediate analysis of natural killer cells and CD4+/CD8+ ratio. The rest of the blood in the third vacutainer was immediately centrifuged and the plasma was stored at -70°C until assayed (Hooper et al, 1993) for glutamine and glutamate.

Group 1:

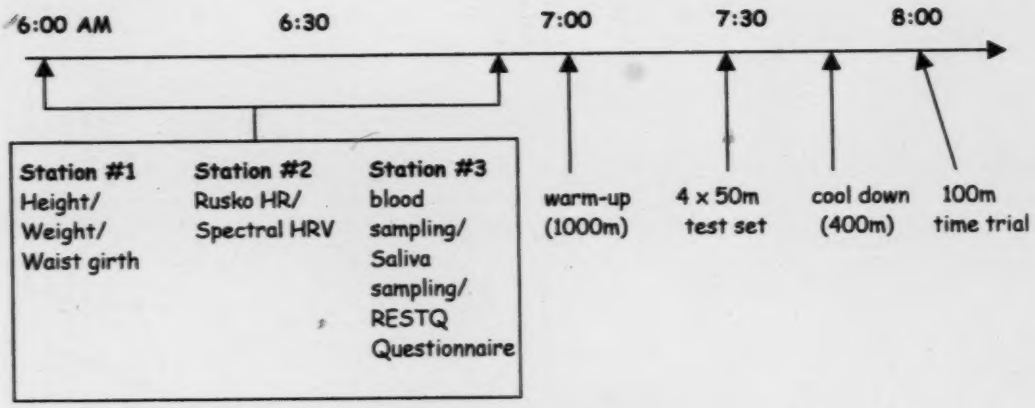

Figure 3.2a. Time Line of Morning Testing Session (Group 1)

Group 2:

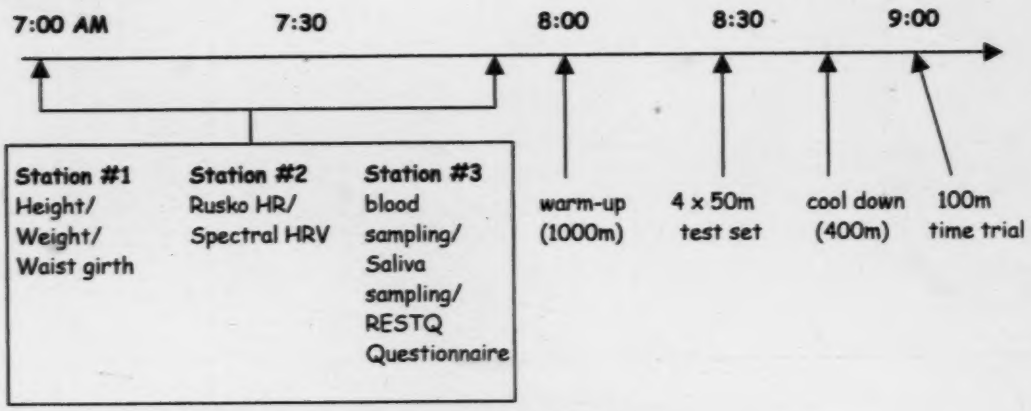

Figure 3.2b. Time Line of Morning Testing Session (Group 2)

C) *AFTERNOON TESTING SESSION (Figure 3.3):*

Testing was also completed in the afternoon between 3 and 4 PM. This testing session occurred on the same day as the morning testing session. The reason for two

sessions was that all of the testing could not be completed in a single session, and rest was needed between the swimming test sets.

At 3:00PM all of the subjects were given a standardized 1000m warm-up which was the same as the morning warm-up (Appendix G). Once warm-up was completed, the 5 x 200m incremental swim test was performed. Once this test was completed the athletes continued with their normal training session.

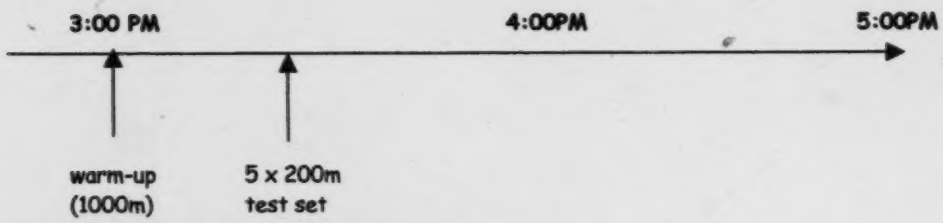

Figure 3.3. Time Line of Afternoon Testing Session

STUDY LOCATION:

The testing was performed at the University of Alberta and at the Kinsmen Sports Center, both of which are located in Edmonton. Swim training was performed in the 25m and 50m pool at the Kinsmen Sports Center as well as at the West pool at the University (25m). The morning testing session was conducted at the University of Alberta. Station #1 (height, weight, and waist girth) was completed in the undergraduate exercise physiology lab in the Physical Education building. Station #2, which included the dynamic postural heart rate test (Rusko), and spectral heart rate variability analysis were performed in the anatomy lab on the fourth floor of the Physical Education building. The blood collection, saliva sampling, and RESTQ questionnaire (station #3) were completed in the Women's Health Lab, which is also in the Physical Education building. Venipuncture was performed in

this room in order to comply with health regulations, as there was a level 2 biosafety permit for this room from the University of Alberta Environmental Health and Safety office. The morning swimming tests were conducted in the University of Alberta West pool (25m pool), and the afternoon swim testing took place in the 50m pool at the Kinsmen Sports Center. The majority of lab work was completed in biochemistry labs in the Agriculture-Forestry building at the University of Alberta. Specifically, S-IgA, glutamine/glutamate, NK cells and CD4+/CD8+ ratio assays were performed in the nutrition labs. The determination of cortisol concentration was completed in a biochemistry lab in the Physical Education building. CBC and differential and iron profile were analyzed by staff in the Dynacare Kasper laboratory in the University of Alberta Hospital.

MEASURES AND METHODS:

A) SUBJECT CHARACTERISTICS:

1. Height:

The subjects' height was measured using a set square and measuring tape to the nearest 0.1 cm with the swimmers in their bathing suits with shoes removed. The swimmers were instructed to look forward, stand as tall as possible, and take a deep breath and hold, after which the measurement was taken.

2. Weight:

Body weight was measured with subjects in their bathing suits with shoes removed, on a beam balance scale to the nearest 0.1 Kg. The scale was calibrated with a set of known weights prior to each testing session.

3. Waist Circumference:

The swimmers were instructed to stand straight, looking forward, with their weight evenly distributed. Waist circumference was measured by positioning the girth tape at the level of noticeable waist narrowing (or in the case of no noticeable narrowing, at the level of the umbilicus), and the measurement was then taken at the end of a normal expiration. The value was recorded to the nearest 0.5cm.

4. 3-day dietary record:

A 3-day dietary record was used to assess the subjects' daily nutrient intake. The energy intake was recorded for the 3 days prior to the testing on October 15th (during a regeneration microcycle) and again before the testing on November 12th (during a crash microcycle). The dietary intake was recorded on two weekdays, and one weekend day. The Food Processor® program was used to quantify daily grams of protein, fat and carbohydrate. Total energy intake was also calculated, along with the percentage that each macronutrient (protein, fat and carbohydrate) contributed to energy intake.

B) TRAINING:

1. Training Record:

The swimmers kept a daily training log of training volume (distance swum), as well as daily subjective ratings of training intensity and fatigue (recorded on a 7-point scale). Time spent doing dryland activities was also recorded (Appendix E). As well, the coaches kept a detailed training log of the volume completed by the swimmers.

C) *HEMATOLOGICAL MEASURES:*

1. CBC and Differential:

In order to perform the complete blood count and differential, blood was collected during the morning testing sessions in a 5mL vacutainer which contained K_3 EDTA (Becton Dickinson, Franklin Lakes, NJ). The subjects were in a fasted state for 8 hours when the blood was taken. Once all of the blood was obtained from each of the subjects, it was stored in a biohazard container packed with ice, and promptly delivered to the Dynacare Kasper Laboratory at the University of Alberta Hospital for analysis by their trained staff. Automated cell counters were used to determine red cell indices, and white cell counts with full differentials.

2. Iron Profile:

For the iron profile determination, blood was collected in the same manner as for the CBC and differential. However, the blood for this analysis was collected in a 4 mL vacutainer that contained SST● Gel and Clot Activator (Becton Dickinson, Franklin Lakes, NJ). Similarly, when all of the blood had been obtained, the vacutainers were delivered to the Dynacare Kasper Laboratory at the University of Alberta Hospital for determination of iron, total iron binding capacity, saturation index, and ferritin by their staff.

D) *IMMUNOLOGICAL MEASURES:*

1. NK cells and CD4+/CD8+ Ratio:

For the purpose of the NK cells and CD4+/CD8+ analysis, 5 ml of blood was collected in a BD Vacutainer™ that contained sodium heparin (Becton Dickinson, Franklin Lakes, NJ).

Immediately after all of the blood samples were collected, the vacutainers were transported in a biohazard container packed with ice to a biochemistry lab in the Agriculture, Food and Nutritional Science department at the University of Alberta. The blood was then spun at 3000rpm for 10 minutes. The plasma was removed and frozen at -70°C for later analysis of glutamine and glutamate. The remaining formed elements were then used in an immunofluorescence (IF) assay to determine the proportion of NK cells and CD4+/CD8+. The precise protocol for the IF assay can be found in Appendix H. Upon completion of the assay, flow cytometric analysis was performed. Flow cytometric analysis can identify specific cell surface antigens (clusters of differentiation, CD) which can be detected with monoclonal antibodies (Nicoll et al, 2001). The NK cells and CD4+/CD8+ analysis was performed by the primary researcher for this study along with the help of the lab technician who was very familiar with the protocol.

2. Glutamine / Glutamate:

As was mentioned above, plasma was used for the determination of glutamine and glutamate. The plasma was stored at -70°C until analysis. Both glutamine and glutamate were analyzed using high performance liquid chromatography (HPLC). This measurement technique uses pre-column derivatisation to measure all major tissue free amino acids, including glutamine and glutamate (Rowbottom et al, 1996). The procedure for this analysis has been described by Sedgwick et al (1991). Only single measures were performed due to the cost of analysis. The analysis of glutamine and glutamate was performed by the primary researcher of this study along with the lab technician in the HPLC laboratory.

3. Salivary Immunoglobulin A:

S-IgA was analyzed from the morning saliva samples that each subject gave on each testing day. The saliva was collected using salivettes (Sarstedt Inc.) which consisted of a cotton swab and a plastic tube. All of the morning saliva samples were kept frozen at -20°C until assayed. The evening before the analysis, the saliva samples were removed from the freezer and placed into a fridge to thaw. The next morning, S-IgA was determined through an indirect enzyme immunoassay kit (Salimetrics, catalog No. 1-1602/1-1612). The primary researcher for this study and a lab technician who was familiar with the protocol performed the analysis. Two kits were needed to run all of the samples in duplicate. The instruction manual for the kits provided step by step procedures. Upon completion of the assays, both of the 96 well plates were read on a plate reader at 450nm. The concentrations of the unknown samples were determined through interpolation using a 4 parameter sigmoid minus curve fit. The concentrations of the samples were multiplied by 5 in order to obtain the final concentrations of S-IgA in μg/mL.

E) HORMONAL MEASURE:

1. Cortisol:

According to Duclos et al (1997), saliva assays have a great ability to detect a cortisol increase. Therefore these researchers strongly support the use of saliva to study H-P-A physiology. From a more practical standpoint, Duclos et al also suggest that the use of saliva helps to minimize sampling stress. Based on these recommendations, cortisol was determined from the saliva of the subjects for this study.

As previously described, each subject provided two saliva samples (one between 4-5 PM and again between 8-10 PM) on the night preceding the testing day. As well, subjects gave one saliva sample during the morning on the test day. Therefore, each subject should have had a total of 15 saliva samples throughout the study. Each saliva sample was collected in a salivette (Sarstedt Inc.). All subjects were provided with take-home instructions on how to properly give their saliva samples (Appendix I). Saliva samples were stored at -20°C until assayed.

The evening before the saliva tubes were to be analyzed, the samples were removed from the freezer and placed into a fridge to thaw. The next morning, salivary cortisol was determined through a Gamma Coat [^{125}I] Cortisol radioimmunoassay (RIA) kit (DiaSorin, Stillwater, Minnesota, USA, catalog No. CA-1529). According to Viru & Viru (2001), RIA is the most valid method for hormone determination, as this method has high analytical sensitivity and specificity. The RIA was performed by the primary researcher for this study with the guidance of an associate professor in the Faculty of Physical Education and Recreation. Procedures were obtained from the Clinical Assays ™ Gamma Coat ™ Cortisol ^{125}I RIA kit instruction manual. Basically, the standards and the unknown samples were incubated with cortisol tracer in antibody-coated tubes where the antibody was immobilized onto the lower wall of the GammaCoat tube. After incubation the contents of the tubes were decanted and the tube was counted on a gamma counter (Diasorin, Gamma Coat ™ Cortisol ^{125}I RIA kit instruction manual, 2002).

However, changes had to be made based on the fact that saliva was being used as opposed to serum, plasma or urine. Specifically, the 5 serum standards that were used to make the standard curve had to be diluted, so that the concentrations of the unknown

cortisol samples would fall within the standard curve. Unknown cortisol values for each sample were interpolated from the standard curve.

For this study, duplicate measures were performed on all samples. As well, for samples whose coefficient of variation was greater than 10%, duplicate measures were run again on a different day. Saliva samples were stored in a fridge for three days before the RIA was run again. The Gamma Coat [^{125}I] Cortisol radioimmunoassay kit that was used for the first analysis was also used the second time. The final cortisol concentrations for the samples that were run again were selected based on the best coefficient of variation (%) for either of the assays.

F) CARDIOVASCULAR MEASURES:

1. Dynamic Postural Heart Rate Test (Rusko):

This test required the subjects to lie supine for 8 minutes and then shift to an upright position for 2 minutes, therefore the test took a total of 10 minutes to complete. Heart rate response to this posture change was monitored every 5 seconds with Polar® heart rate monitors with downloadable memories. Testers also recorded heart rate measurements for the last 2 minutes of lying, 15 seconds after standing, as well as the last 30 seconds of standing (Appendix J). Only these time periods were noted as they provide the most valuable information regarding the autonomic nervous system's response to orthostasis. Various testers recorded the results of the Rusko Heart Rate Test, however all assistants were provided with an instruction session prior to performing the testing on the subjects.

2. Spectral Heart Rate Analysis:

This test took a total of 6 minutes to complete per subject. One at a time, subjects lay comfortably on a gym mat on the floor and were hooked up to the OmegaWave Sport Technology System ©. Both of the researchers who performed this test were trained on the OmegaWave Sport Technology System. Using this system, heart rate variability evaluation was made based on information from a standard, three-lead electrocardiogram (ECG). Specifically, electrodes were clipped to each of the subject's limbs (i.e. cable R was attached to the subject's right leg, cable L to the left leg, cable F to the right arm, and cable N to the left arm). See Figure 3.4 for a diagram of the set-up. The OmegaWave Sport Technology System also provided information on the functioning of the subject's cardiovascular system by reporting numeric values for the following 5 parameters: 1.) Activity of vagus regulation mechanisms; 2.) Activity of sympathetic regulation mechanisms; 3.) Tension index; 4.) Share of the aperiodic influences; 5.) Standard deviation of the aspirate waves

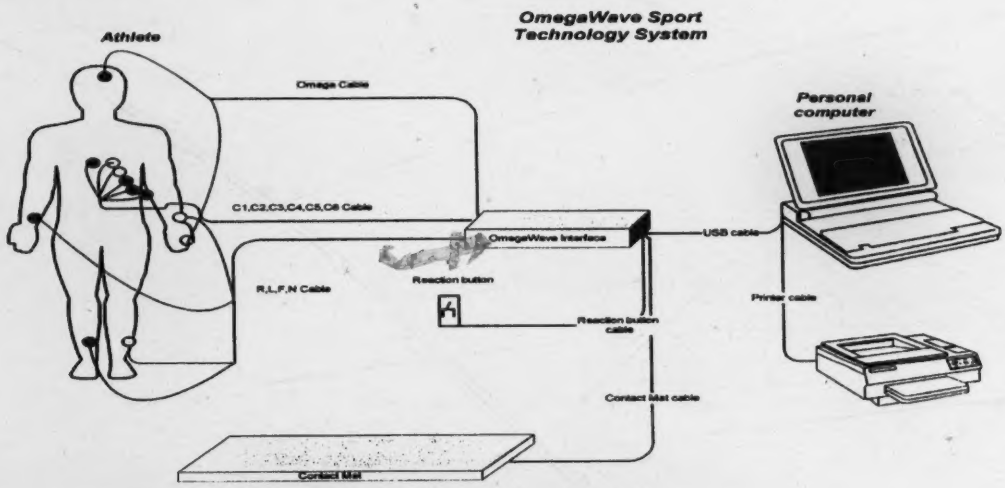

Figure 3.4. Diagram of the OmegaWave Sport Technology System.
Note: for heart rate evaluation, only the R, L ,F and N cables are attached to the athlete.
(Obtained from the OmegaWave Operating Manual, 2001)

G) *PSYCHOLOGICAL MEASURE:*

1. RESTQ Questionnaire:

Although the results for the RESTQ Questionnaire are not discussed in this study, the questionnaires were completed for analysis at a later date. The procedure for the questionnaire involved having the subjects sit quietly at a table for 15 minutes while they answered the 76 questions using a pencil or pen. The questionnaire was designed specifically for athletes to examine stress and recovery (Kellemann et al, 2001).

H) *PERFORMANCE MEASURES:*

1. 5 x 200m Swim Test:

The standard protocol was to swim a graded incremental test through the five 200m swims of either freestyle or backstroke. Each 200m swim was on 4 minutes, therefore the entire test took 20 minutes to complete. The swimmers were instructed to start each 200m from a push in the water, and to even split each 200m swim (i.e. swim the first 100m at the same speed as the second 100m). Heart rate measurements were taken immediately at the end of each 200m, to determine cardiovascular responses to increased swimming speeds (Pyne et al, 2000b). Heart rate measurements were obtained from Polar® heart rate monitors. Athletes were instructed to swim the intervals such that their heart rate progressed from approximately 60 beats below their maximal heart rate for the first swim to 20 beats below their maximal heart rate for the last swim (Norris & Smith, 2001, unpublished). Therefore, their heart rate increased by approximately 10 beats/minute for each 200m swam. The data obtained was used to create a heart-rate velocity curve. A computer software program designed by Norris & Smith was used to graph the results. By

extrapolation of the slope of the graph, a prediction was made for each swimmer's 400m swim time. The swimmers participating in this study were very familiar with this test.

2. 4 x 50m Swim Test:

The protocol for the test involved swimming 4 x 50m's of the athlete's main stroke on a 2:00 interval. Therefore, the entire test took 8 minutes to complete. The swimmers were instructed to hold the best average that they could for the 4 swims (i.e. each repeat was to be a maximal effort). The set was swum from a push for all of the 50m's. This set was used to provide a prediction of the athlete's 100m speed. This prediction was done by simply doubling the average of the 50m's (Norris & Smith, 2001, unpublished).

3. Time Trials:

The 100m time trial was swum at maximal effort in the athlete's main stroke. Swimmers started from a dive, on the coaches "go". The two coaches involved in this study timed the event. These two coaches, both of whom are Nationally certified by Swimming/Natation Canada, were also responsible for timing the 5 x 200m test set and the 4 x 50m test set.

4. Competitions:

Results from the major competition in November (either the Canadian National Swimming Championships or the Prairie Winter Invitational) were analyzed based on event and time achieved. The percent of personal best time was calculated for each subject's best three events.

STATISTICAL ANALYSES:

To determine baseline values for each subject, the results from testing on September 9th and September 12th were averaged in order to obtain a starting value. Once that was completed, differences between testing times (baseline, build, crash and taper) for each variable were analyzed using an analysis of variance (ANOVA) with repeated measures. As well, due to the large number of variables being analyzed and the number of multiple comparisons, a Bonferroni correction factor was used to establish the appropriate alpha level (Meyers & Wells, 1995).

If the repeated measures ANOVAs determined that there was a significant main effect of time for a variable, *post hoc* analysis was performed using multiple t-tests to determine the specific time points where the differences existed. For all *post hoc* analyses, an alpha level was set *a priori* at $p < 0.05$.

Unfortunately, for a select number of variables, the repeated measures ANOVA produced inaccurate results due to a violation in Mauchly's test of sphericity. Therefore, for these variables, a multivariate test was run to determine whether there was a significant main effect of time. If the multivariate test revealed significance, then *post hoc* analysis was performed using a Scheffe test. For this *post hoc* analysis, significance was also set at $p < 0.05$.

In addition to the group data, individual results were also considered. Upon completion of the study, subjects were split into three groups based on their performance at their major competition. Group one represented the individuals that performed 100% best times in their main events at the competition (RESPONDERS). Group two signified the individuals that achieved 97% -99% of their best times in their main events, and group

three (NON-RESPONDERS) represented the athletes who achieved times in their main events that were worse than 97% of their best times. The athletes from groups one and three were compared using descriptive statistics and graphs for each variable to visually determine whether differences existed between these two groups.

Furthermore, a case study approach was used to examine one individual athlete based on her outlying data, and her profound under-performance at the Canadian National Championships. Her data has been graphed, and in many situations her results have been compared to the group means.

CHAPTER FOUR: RESULTS

OVERVIEW:

This chapter initially examines the mean results for the 14 subjects for each of the variables. Following the group analysis, descriptive statistics are used to examine the subjects who performed well (RESPONDERS, n=3) at their major competition (100%+ best times), and the subjects who performed poorly (NON-RESPONDERS, n=3) (less than 97% of their best times in their main events). Finally, the results for the case study subject are examined.

In this chapter, the terms "baseline", "build", "crash" and "taper" have been used to identify the different testing periods. The baseline tests were performed before the start of the training season (mean of September 9th and 12th). The build test was performed on October 15th, following a training period of moderate volume and intensity. The crash test occurred on November 12th, following a training phase characterized by a considerable increase in volume and highly intense training sessions. Finally the taper test occurred on November 25th, which was only 3 days prior to the start of the subjects' major competition.

GROUP RESULTS:

A) *SUBJECT CHARACTERISTICS:*

Eight female and six male competitive swimmers (aged 16-23 years; $\bar{x}$ = 18.3 years ± 0.6) completed this study. Subject characteristics were obtained at each testing session for all 14 subjects. Measures collected included body height, weight, and waist circumference. Body mass index (BMI) was calculated using the formula Kg/m^2 (Table 4.1).

Table 4.1 Anthropometric Measures for Male and Female Subjects at Each Test

Measure	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)	
	male n=6	female n=8	male n=6	female n=8	male n=6	female n=8	male n=6	female n=8
Height (cm) *°	188.4	175.4	188.0	174.9	188.3	175.1	188.2	175.0
SE	3.3	1.5	3.3	1.5	3.2	1.4	3.3	1.4
Range	175.9-195.3	167.6-182.6	175.6-194.9	167.0-182.6	175.9-195	167.6-182.2	175.3-195	167.6-182.2
Weight (Kg) *	82.9[a]	62.4	80.7[b]	63.2	79.4	62.8	78.9[ab]	62.5
SE	3.4	1.1	4.2	1.2	3.8	1.3	4.0	1.3
Range	74.4-96.7	58.9-67.9	68.0-96.3	59.5-69.3	66.8-93.2	59.4-69.4	66.7-94.3	58.9-69.5
Waist (cm) *	82.0[a]	69.9	81.3[bc]	71.2	79.5[b]	70.5	79.0[ac]	70.1
SE	1.9	1.5	2.1	1.4	1.7	1.6	1.9	1.6
Range	75.5-89.8	65.0-76.5	74.5-90.0	66.5-78.0	73.0-86.0	65.0-78.5	73.5-87.0	64.4-79.0
BMI (Kg/m^2) *	23.3[a]	20.3	22.8[bc]	20.7	22.4[b]	20.5	22.2[ac]	20.4
SE	0.6	0.4	0.7	0.5	0.6	0.5	0.6	0.5
Range	20.7-25.3	19.0-22.6	20.4-25.4	19.5-23.0	20.1-24.5	19.2-23.2	19.9-24.8	19.2-23.2

* = significant main effect of time at $p < 0.05$ for the males
° = significant main effect of time at $p < 0.05$ for the females
matching alpha characters denote significance at $p < 0.05$ between test periods

A repeated measures ANOVA was performed on all of the subject characteristic measures for males and females separately. For these measures, significance was set *a priori* at $p < 0.05$. Height results for both the males and females showed a significant effect of time, $F(3,15)=3.57$, $p=0.04$ and $F(2.7,19.2)=4.898$, $p=0.012$ respectively. *Post hoc* analysis, however, revealed that no significant differences actually existed between any of the testing periods for males or females. Therefore, the significant effect of time in general can be determined as meaningless as differences were not observed between specific time points.

Weight results demonstrated a significant main effect of time for the males, $F(3,15) = 10.79$, $p = 0.000$, but not for the females. *Post hoc* analysis determined that male

athletes were statistically significantly lighter at the taper test compared to the baseline test period ($p = 0.008$), and lighter at the taper test compared to the build test ($p = 0.013$).

Waist circumference results also showed a significant effect of time for the males, $F(3,15)=15.204$, $p=0.000$, but not for the females. *Post hoc* analysis demonstrated that waist circumference in males decreased in size with each test period. Waist circumference was statistically significantly decreased from the baseline test to the taper test ($p=0.001$), from the build test to the crash test ($p=0.053$) and from the build test to the taper test ($p=0.005$) for the male subjects.

Further, BMI results showed a similar trend. BMI results were significantly different across time for the males, $F(3,15)=8.541$, $p=0.002$, but not for the females. *Post hoc* analysis for BMI for the males showed a significant decrease between the baseline test and the taper test ($p=0.014$), between the build test and the crash test ($p=0.045$) and between the build test and the taper test ($p=0.005$).

B) *ENERGY INTAKE:*

Mean 3-day dietary intakes were calculated for each subject at two time periods during the course of the study. The first 3-day dietary record was performed immediately prior to the build test (October 10-12th), and the second 3-day dietary record was completed immediately prior to the crash test (November 7-9th). Means were calculated for males and females separately and are shown in Table 4.2.

Table 4.2 Results from the 3-day Dietary Records (mean ± SE)

MEASURE	BUILD (October 10-12th)		CRASH (November 7-9th)	
	MALES n=6	FEMALES n=8	MALES n=6	FEMALES n=8
Calories (Kcal)	4283	3201	3717	2559
SE	483	280	492	71
Range	2894-6003	2495-4547	2324-5175	2190-2881
Protein (g)°	180	112[a]	155	84[a]
SE	20	12	20	7
Range	108-243	73-162	80-213	53-114
Carbohydrate (g)°	552	444[a]	462	361[a]
SE	56	34	49	7
Range	394-788	384-674	345-676	339-390
Fat (g)	155	112	142	89
SE	23	24	28	8
Range	80-225	61-273	58-218	66-127
Protein (%)	17	14	17	13
SE	1	1	1	1
Range	13-21	11-19	14-21	8-18
Carbohydrate (%)	51	56	51	56
SE	1	4	3	2
Range	45-54	35-65	41-63	47-64
Fat (%)	32	30	32	31
SE	2	4	3	2
Range	25-37	22-53	22-40	25-41

° = significant main effect of time at $p < 0.05$ for the females
matching alpha characters denote significance at $p < 0.05$ between test periods

A repeated measures ANOVA was conducted for each of the dietary variables for males and females separately. Based on the results from the ANOVAs, no statistically significant differences were found for the males for any of the nutrients. For the females, a main effect of time was observed for protein, $F(1,7)=13.689$, $p=0.008$, and also for carbohydrate, $F(1,7)=6.928$, $p=0.034$, but not for any of the other variables. For both protein and carbohydrate, the values were significantly lower the second time the dietary record was performed compared to the first time.

C) *TRAINING:*

The subjects involved in this study were chronically trained. Typically, each athlete trained 6 days/week and attended 5 -8 training sessions. Over the course of this 10 week study, each subject recorded their training history in a daily training log book, noting the volume of each practice (distance swum), the time spent doing dryland activities, and subjective ratings of training intensity and fatigue (recorded on a 7-point scale). As well, subjects were asked to mention the occurrence of illness as well as the presence of menstruation (for the females).

In terms of training volume, the subjects in this study began with low volume training. For week one the mean swim volume was 12 Km/week, and increased to 16.9 Km/week for week two. The volume was then substantially increased for weeks 3 to 5 as the volumes ranged from 30.6-39.1 Km/week. In week 6, the volume decreased slightly to 32.8 Km. Weeks 7 and 8 then signified the period of intensified training as the volume increased to 44.7 and 44.9 Km/week respectively. For the final two weeks of the study, the weekly swim volume was significantly reduced to 36.4 Km and 30.0 Km in weeks 9 and 10 respectively (see Table 4.3 and Figure 4.1). For all of the subjects, the last week to two weeks of the study were designated as a taper period in order to prepare for the major competition. The length of the taper varied for each individual athlete.

In terms of dryland training, the majority of training was done in the first four weeks of training (Figure 4.2). Specifically, during the first four weeks the average time spent doing dryland activities ranged from 112.8 minutes to 128.9 minutes/week. Subsequently, in week 5, the amount of time spent doing dryland activities was reduced to 65 minutes/week. For the rest of the study, the amount of dryland remained significantly

lower than the first 4 weeks, with the amount of time ranging from 45.7 to 81.9 minutes/week. During weeks 7 and 8, when the swim volume was the highest, the amount of dryland time also slightly increased.

Table 4.3 Weekly Training Record (mean volume, SD and range)

	TRAINING DATES	Swim Volume (Km) n=14	SE/ Range	Dryland time (min.) n=14	SE/ Range
Baseline Test ▶	Week 1: Sept. 16 - 22	12.0	0.7 / 10.5-17.1	128.9	36.1/ 0-375
	Week 2: Sept. 23 - 29	16.9	0.8 / 7.9-21.0	120.6	24.7/ 10-330
	Week 3: Sept. 30 - Oct. 6	30.6	2.5 / 19.0-39.3	112.8	26.2 / 20-370
Build Test ▶	Week 4: Oct. 7 - 13	39.1	2.0/ 26.0-53.0	128.9	16.8 / 45-240
	Week 5: Oct. 14 - 20	37.9	1.8 / 23.7-47.6	65	14.0 / 0-200
	Week 6: Oct. 21 - 27	32.8	2.3 / 14.1-46.7	51.3	11.5 / 0-140
	Week 7: Oct. 28 - Nov. 3	44.7	4.5 / 22.9-74.7	70	15.8 / 20-195
Crash Test ▶	Week 8: Nov. 4 - 10	44.9	4.0 / 6.0-69.5	81.9	14.2 / 15-195
	Week 9: Nov. 11 - 17	36.4	2.6 / 24.8-54.0	58.3	16.7 / 0-180
Taper Test ▶	Week 10: Nov. 18 - 24	30.0	3.5 / 4.4-52.0	45.7	16.0 / 0-165

The subjects in this study also provided a subjective rating of the intensity of each training session, which they recorded in their training log books. One of the coaches also provided weekly ratings of training intensity. The other coach, unfortunately, failed to provide these ratings. The coach who gave the ratings suggested that week 8 was the highest intensity. This was also the week of highest swimming volume. Similarly, many of

the athletes reported high ratings of intensity during weeks 7 and 8 when the volume was very high (see Table 4.4, Figure 4.3).

With respect to the subjective rating of training fatigue in the log books, the fatigue measure was highest during week 7 (see Table 4.4, Figure 4.4).

Table 4.4 Subjective Ratings of Training Intensity and Fatigue

	TRAINING DATES	TRAINING INTENSITY			FATIGUE (n=14)	
		coach's rating (n=1)	subjects' rating (n=14)		subjects' rating	
		Rating	Mean	SE/ Range	Mean	SE/ Range
Baseline Test ▶	Week 1: Sept. 16-22	2	2.4	0.2/ 1.4-3.8	2.6	0.2/ 1.6-4.0
	Week 2: Sept. 23-29	2	3.8	0.2/ 1.5-5.3	3.6	0.2/ 2.5-4.4
	Week 3: Sept. 30-Oct. 6	4	3.6	0.2/ 2.3-4.6	3.7	0.2/ 2.5-4.9
Build Test ▶	Week 4: Oct. 7 - 13	4	3.9	0.2/ 3.1-5.0	4.0	0.2/ 2.9-5.3
	Week 5: Oct. 14 - 20	5	4.5	0.2/ 3.2-5.4	4.6	0.2/ 2.6-6.0
	Week 6: Oct. 21 - 27	6	4.1	0.3/ 2.4-6.1	4.3	0.3/ 2.4-6.2
	Week 7: Oct. 28-Nov. 3	4	4.5	0.1/ 2.8-4.5	5.3	0.2/ 2.4-5.4
Crash Test ▶	Week 8: Nov. 4 - 10	7	4.4	0.2/ 3.2-5.0	4.7	0.2/ 2.8-6.2
	Week 9: Nov. 11 - 17	6	4.5	0.2/ 3.6-5.9	4.4	0.2/ 3-6.3
Taper Test ▶	Week 10: Nov. 18 - 24	4	4.0	0.3/ 1.4-5.5	4.2	0.3/ 2.0-6.4

1=very, very low; 2=very low; 3=low; 4=moderate; 5=high; 6=very high; 7=very, very high

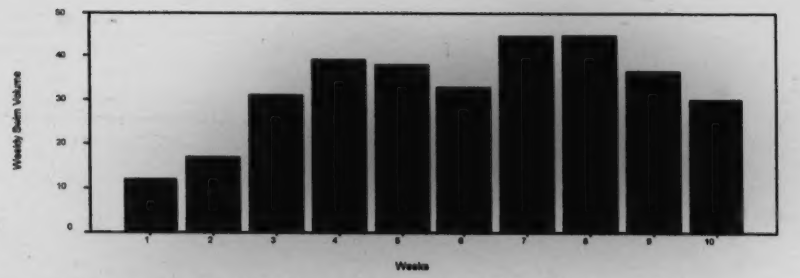

Figure 4.1 Average Swim Training Volume (Km) by Weeks

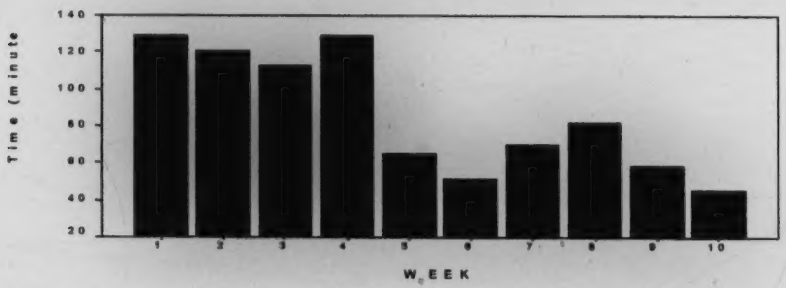

Figure 4.2 Average Time Spent (minutes) doing Dryland Activities by Weeks

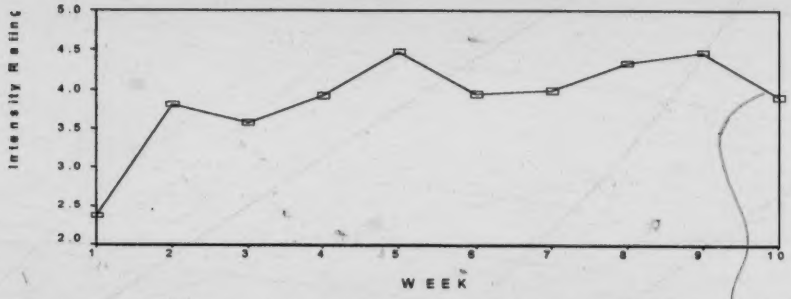

Figure 4.3 Subjective Ratings of Training Intensity by Weeks

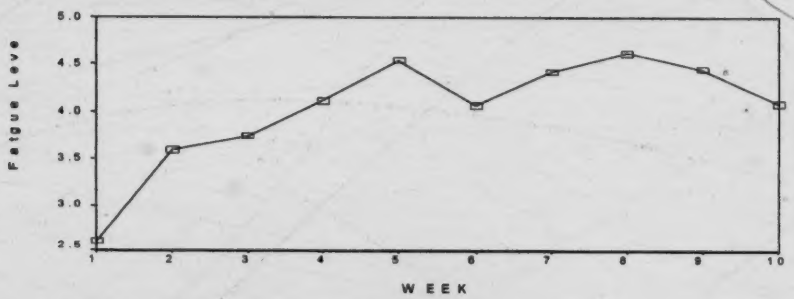

Figure 4.4 Subjective Ratings of Fatigue by Weeks

Further, Table 4.5 reports the frequency of illnesses among all subjects during the 10 week training period. And finally, Table 4.6 notes the frequency of menstruation for the female athletes.

Table 4.5 Frequency of Illnesses Recorded in Training Log Books

Subject #	Frequency of illness (workouts)	Total # of Workouts recorded	Percentage of Workouts ill (%)
01	19	77	24.7
02	4	62	6.5
03	4	77	5.2
04	7	71	9.9
05	0	71	0
06	9	71	12.7
07	1	66	1.5
08	14	76	18.4
09	10	74	13.5
10	12	57	21.1
11	19	57	32.2
12	0	80	0
13	9	78	11.5
14	7	71	9.9
MEAN	8.2	70.6	11.9

Table 4.6 Menstrual Cycle Frequency over the Course of the Study (68 days)

Subject #	Frequency of menstruation (days) during study	Number of cycles recorded	% of days menstruating throughout study	Mean length of period (days)*
01	10	2	14.7	5
02	7	2	10.3	3.5
08	19	3	27.9	6.3
09	12	2	17.6	6
10	12	2	17.6	6
11	9	2	13.2	4.5
13	10	2	14.7	5
14	8	2	11.8	4
MEAN	10.9	2.1	16.0	5.0

Note: *mean length of period is estimated from training log book

D) HEMATOLOGICAL MEASURES:

Red blood cell indices examined included hemoglobin, (Hb) red blood cell count, hematocrit (Hct), mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) (Table 4.7). Iron, ferritin and total iron binding capacity were also investigated at each testing session (Table 4.8). Repeated measures ANOVAs were run for each of these hematological measures. Since there were 9 hematological variables, significance was set *a priori* at $p<0.006$ using the Bonferroni correction (0.05/9).

1. Red Blood Cell Indices:

Table 4.7 Red Blood Cell Markers

MEASURE	Baseline (Sept. 9 & 12) n=14	Build (Oct. 15th) n=14	Crash (Nov. 12th) n=14	Taper (Nov. 25th) n=14
Hemoglobin (g/L)	148.4	148.0	148.8	150.4
SE	3.6	3.7	3.3	2.9
Range	133 - 182	126-177	132-174	138-173
Red Blood Cell Count (10^{12}/L)*	4.88^{a}	4.80^{b}	4.79^{ac}	4.90^{bc}
SE	0.1	0.1	0.1	0.1
Range	4.29 - 5.86	4.07-5.66	4.19-5.51	4.42-5.64
Hematocrit (L/L)	0.43	0.43	0.43	0.44
SE	0.009	0.01	0.009	0.008
Range	0.40 - 0.53	0.37-0.51	0.38-0.50	0.41-0.51
Mean Cell Volume (fL)*	88.68^{abc}	89.86^{a}	89.71^{b}	89.64^{c}
SE	0.6	0.7	0.6	0.7
Range	85 - 94	86-95	86-95	85-94
MCHC (g/L)*	343.3^{a}	342.9^{b}	346.0^{abc}	342.3^{c}
SE	1.0	1.3	1.0	
Range	337 - 349	336-352	338-350	

* = significant main effect of time at $p < 0.006$
matching alpha letters denote significance at $p < 0.05$ between test periods (based on *post hoc* analysis)

The Hb results revealed no significant effect of time. However, from a physiological perspective, changes did occur across testing periods. As can be seen from Figure 4.5, mean Hb concentrations were at their highest during the taper period.

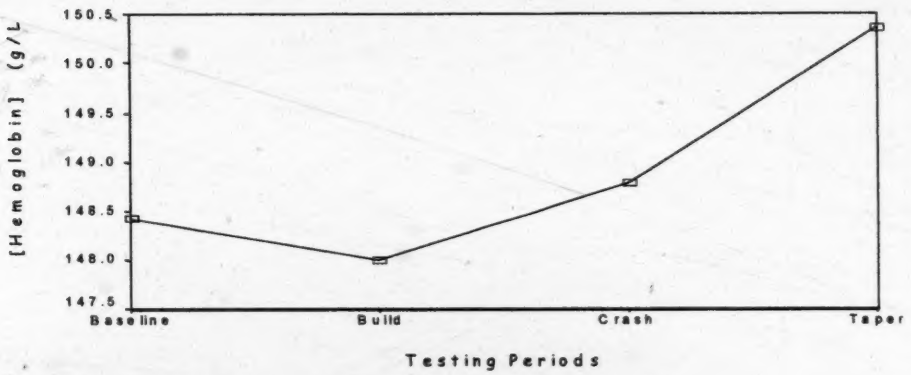

Figure 4.5 Hemoglobin Concentrations Across Testing Periods

Results for the red blood cells showed a significant main effect of time, $F(3,39)=4.780$, $p=0.006$. *Post hoc* analysis determined that differences existed between the baseline test and the crash test ($p=0.044$), between the build test and the taper test ($p=0.026$), and between the crash test and the taper test ($p=0.002$). It can be seen that the red blood cell count was lowest during the crash period and highest during the taper (Figure 4.6).

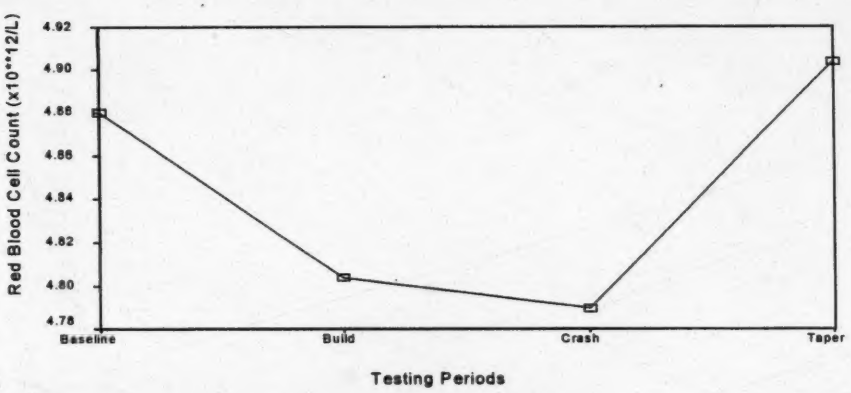

Figure 4.6 Red Blood Cell Count Across Testing Periods

Hct results showed no significant differences across testing times at the level of $p < 0.006$. However, as can be seen in Figure 4.7, Hct values were at their highest during the taper period.

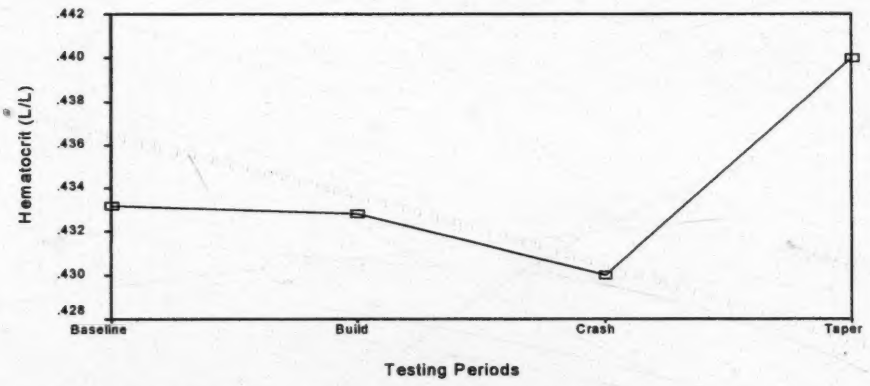

Figure 4.7 Hematocrit Values Across Testing Periods

Results for the MCV were statistically significant across time, $F(3,39)=14.238$, $p=0.000$. Most noticeably, the differences occurred between the baseline test and all of the other testing times (Figure 4.8).

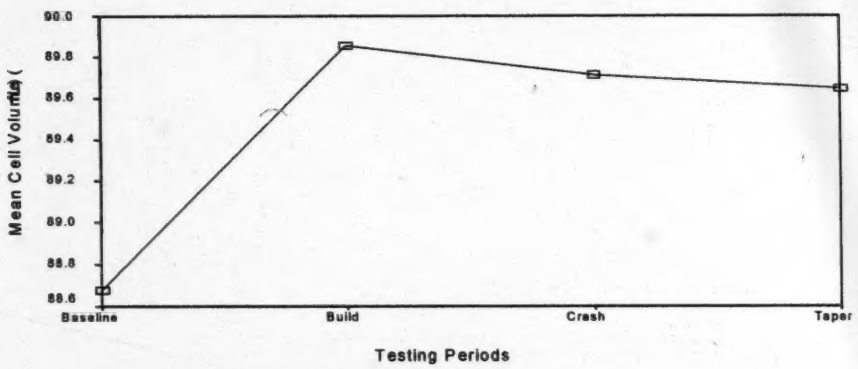

Figure 4.8 Mean Cell Volume Across Testing Periods

Results from the repeated measures ANOVA for the MCHC showed a significant main effect of time, $F(3,39)=6.392$, $p=0.001$. Specifically, *post hoc* analysis identified that the differences existed between the baseline test and the crash test ($p=0.003$), between the build test and the crash test ($p=0.005$) and also between the crash test and the taper test ($p=0.001$). As can be seen from Figure 4.9, it is clear that the highest MCHC in concentration for the group was found during the crash period.

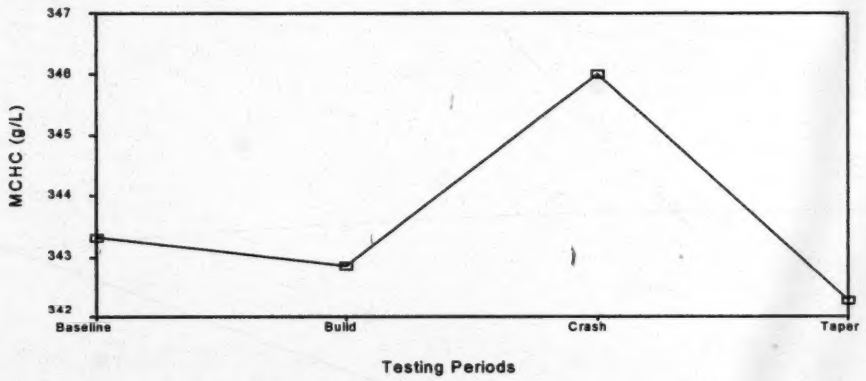

Figure 4.9 Mean Cell Hemoglobin Concentration (MCHC) Across Testing Periods

2. Iron, Total Iron Binding Capacity and Ferritin:

A repeated measures ANOVA was also run for iron and its associated variables including ferritin and total iron binding capacity (Table 4.8).

Table 4.8 Tests of Iron Status

MEASURE	Baseline (Sept. 9 & 12) n=14	Build (Oct. 15th) n=14	Crash (Nov. 12th) n=14	Taper (Nov. 25th) n=14
Iron (μmol/L)*	20.4^{ab}	13.9^{a}	15.3^{b}	16.4
SE	1.7	1.0	2.0	1.6
Range	7-29	9-22	4-33	4-25
TIBC (μmol/L)*	57.9	53.9	57.2	58.1
SE	2.0	2.0	2.5	3.0
Range	44-71	43-71	45-81	46-88
Ferritin (μg/L)*	79.8	45.9	51.2	56.9
SE	11.9	7.3	7.4	14.3
Range	27-173	17-114	15-106	13-226

* = significant main effect of time at $p < 0.006$
matching alpha characters denote significance at $p < 0.05$ between test periods (based on *Post hoc* analysis)

Results from the repeated measures ANOVA for iron demonstrated that there were significant differences across time, $F(3,39)=4.498$, $p=0.008$. *Post hoc* analysis found significant differences between the baseline test and the build test ($p=0.001$) and also between the baseline test and the crash test ($p=0.047$) (Figure 4.10).

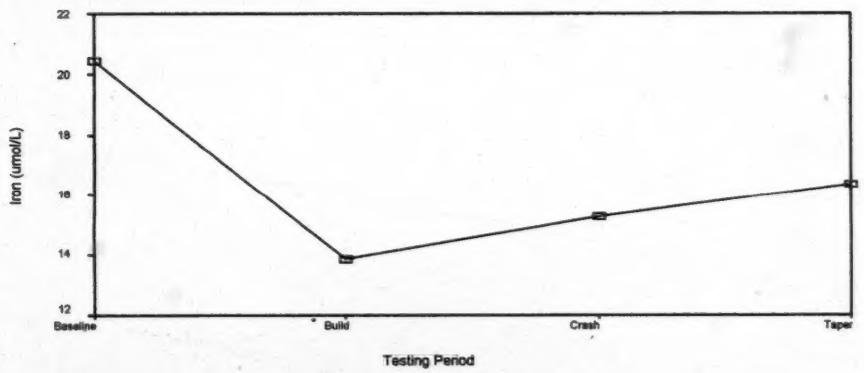

Figure 4.10 Iron Concentrations Across Testing Periods

The results from the repeated measures ANOVA for total iron binding capacity (TIBC) created some problems. The results from the repeated measures ANOVA were inaccurate due to a violation of Mauchly's test of sphericity. Therefore, a multivariate test was run which determined that p=0.000. However, *post hoc* analysis using the Scheffe test revealed that no significant differences existed between any of the time points. Therefore, the differences across time in general are negligible. Figure 4.11 illustrates the mean total iron binding capacity results across testing periods.

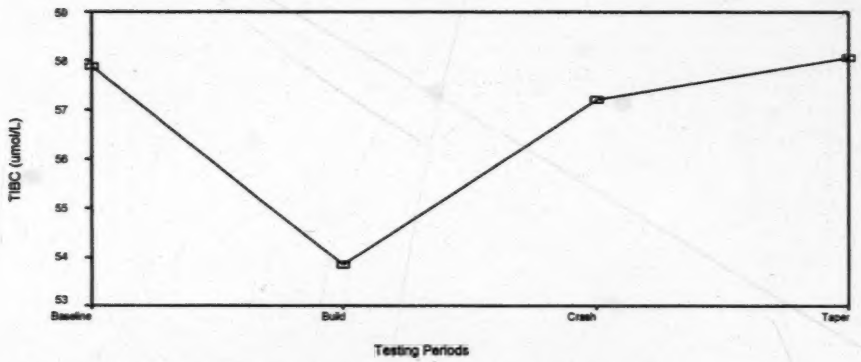

Figure 4.11 Total Iron Binding Capacity Across Testing Periods

A similar problem presented itself for ferritin, and therefore the same analysis procedures were taken. For ferritin, a multivariate test determined that p=0.004. However, *post hoc* analysis using the Scheffe test found no significant differences between any of the specific time points. A graphical representation of the ferritin results can be seen in Figure 4.12

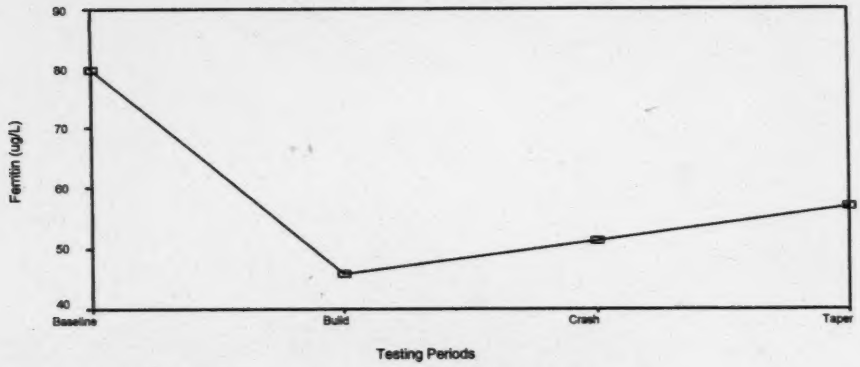

Figure 4.12 Ferritin Concentrations Across Testing Periods

E) *IMMUNOLOGICAL MEASURES*

The immunological tests that were analyzed include white blood cells and differential; natural killer cells (CD16+CD56+); T-cells (CD4+, CD8+ and the CD4+/CD8+ ratio); immunoglobin A; and glutamine, glutamate and the glutamine: glutamate ratio. Results for these variables are discussed in the order mentioned above. For most of these measures, results are provided for all four of the testing sessions. Unfortunately, however, an error in the lab occurred when performing the final assay for the natural killer cells and the T-cells. Therefore, results are missing for these variables for the taper period.

In terms of analysis, repeated measures ANOVAs were run for each of these immunological variables. Further, the Bonferroni correction factor was applied because of multiple comparisons being done. Specifically, there are 15 immunological variables that can be divided into two categories: 1.) variables that are directly related to white blood cells and 2.) other immunological variables. In the first category there were 11 variables including the white blood cells and differential, natural killer cells, T-cells and immunoglobin A. Therefore, significance was set at $p < 0.005$ for these variables using the Bonferroni

correction (0.05/11). The second category contained glutamine, glutamate and the ratio between the two amino acids. For these variables, significance was set at $p < 0.02$ by using the Bonferroni correction (0.05/3).

1. White Blood Cells and Differential:

Mean results for the white blood cells and differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) can be found in Table 4.9.

Table 4.9 Results for White Blood Cells and Differential Across Testing Times

MEASURE	Baseline (Sept. 9 & 12) n=14	Build (Oct. 15th) n=14	Crash (Nov. 12th) n=14	Taper (Nov. 25th) n=14
White Blood Cell (10^9/L)	6.4	6.7	6.2	5.7
SE	0.3	0.5	0.3	0.3
Range	4.7-7.9	4.7-11.2	4.9-9.1	3.8-7.2
Neutrophil (10^9/L)	3.8	3.3	2.9	2.7
SE	0.4	0.4	0.2	0.2
Range	2.3-6.3	1.6-7.5	1.8-5.3	1.4-3.5
Lymphocyte (10^9/L)	2.1	2.6	2.2	2.2
SE	0.1	0.2	0.2	0.2
Range	1.3-3.4	1.8-4.1	0.3-3.4	0.8-3.1
Monocyte (10^9/L)	0.5	0.6	0.7	0.5
SE	0.03	0.04	0.1	0.05
Range	0.3-0.8	0.3-0.8	0.4-1.9	0.3-0.9
Eosinophil (10^9/L)	0.2	0.2	0.2	0.2
SE	0.03	0.03	0.04	0.04
Range	0.0-0.4	0.0-0.5	0.1-0.5	0.1-0.6
Basophil (10^9/L)*	0.008^{abc}	0.06^{ad}	0.05^{be}	0.09^{cde}
SE	0.008	0.01	0.01	0.02
Range	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.2

* = significant main effect of time at $p < 0.005$
matching alpha characters denote significance at $p < 0.05$ between test periods (based on *Post hoc* analysis)

As can be observed from the above Table, white blood cells, neutrophils, lymphocytes, monocytes and eosinophils did not change significantly across the testing periods. The basophil results, however, did reveal a significant main effect of time,

F(3,33)=11.629, p=0.000. *Post hoc* analysis of the basophil results determined that differences occurred between the baseline test and the build test (p=0.002), the crash test (p=0.017), and the taper test (p=0.001); between the build and taper tests (p=0.05), and also between the crash and taper tests (p=0.002). As can be seen in Figure 4.13, basophil concentrations were lowest during the baseline time period, and highest during the taper.

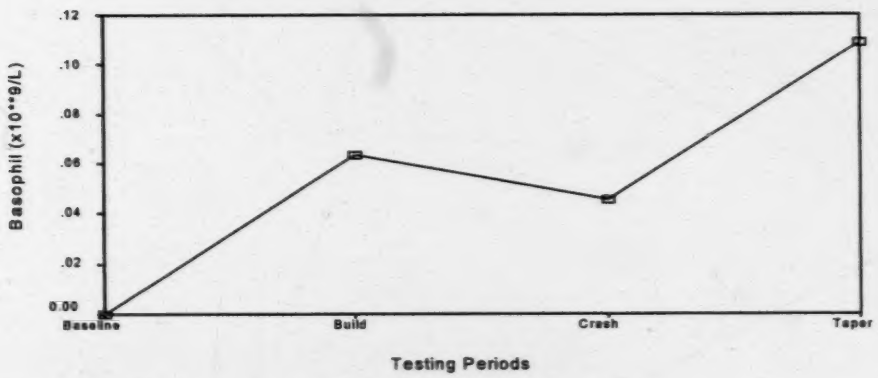

Figure 4.13 Basophil Concentration Across Testing Periods

Despite the fact that statistically significant differences did not exist for white blood cells, neutrophils, lymphocytes, monocytes and eosinophils, the small changes that did occur may be important from a physiological perspective. Therefore, graphs of each of these variables across testing periods can be observed below. As Figure 4.14 illustrates, white blood cell values were at their highest during the build period ($\bar{x}$ = 6.7 x10^9/L) and lowest during the taper ($\bar{x}$ = 5.7 x10^9/L).

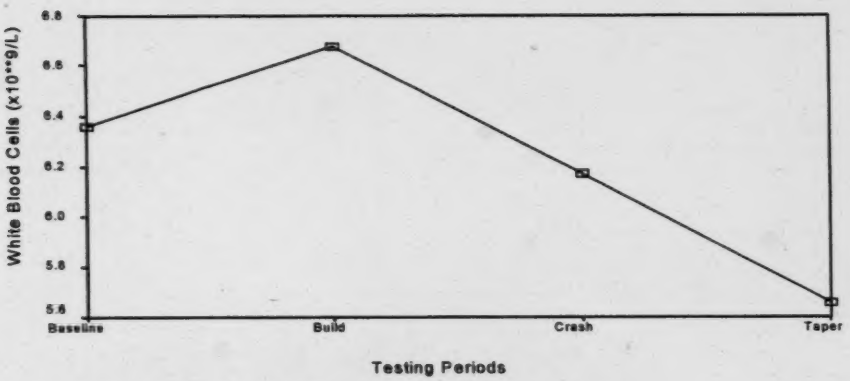

Figure 4.14 White Blood Cell Values Across Testing Periods

In terms of neutrophils, the concentration declined throughout all four of the test periods. This can be observed in Figure 4.15.

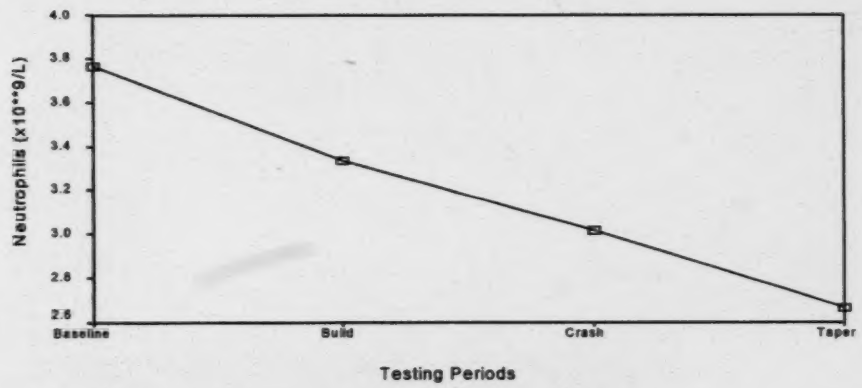

Figure 4.15 Neutrophil Concentrations Across Testing Periods

For lymphocytes, Figure 4.16 demonstrates that the mean concentrations appear to be highest during the build period, and fairly similar at all other testing points.

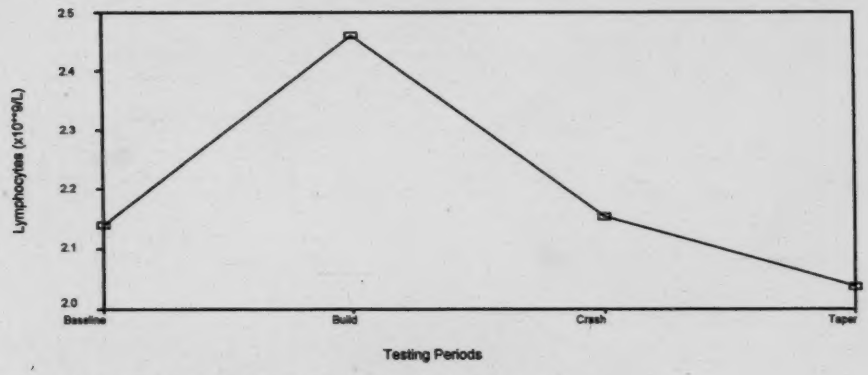

Figure 4.16 Lymphocyte Count Across Testing Periods

Figure 4.17 shows the changes in monocyte concentrations across testing periods. The graph illustrates that the monocyte concentrations were at their highest during the crash period.

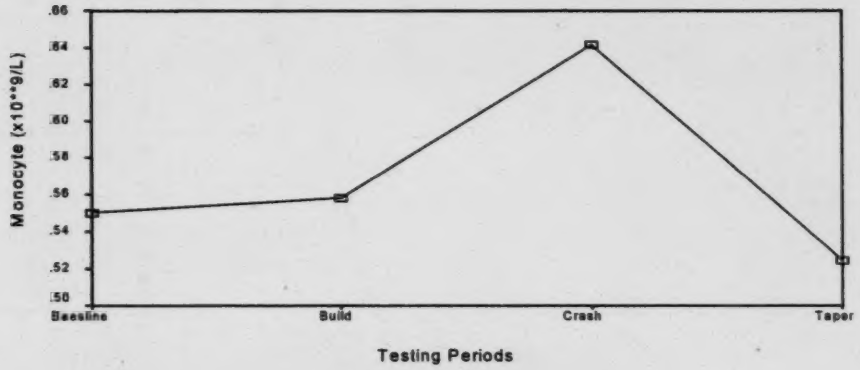

Figure 4.17 Monocyte Concentration Across Testing Periods

And finally, Figure 4.18 demonstrates how the eosinophil concentration increased throughout the four test periods.

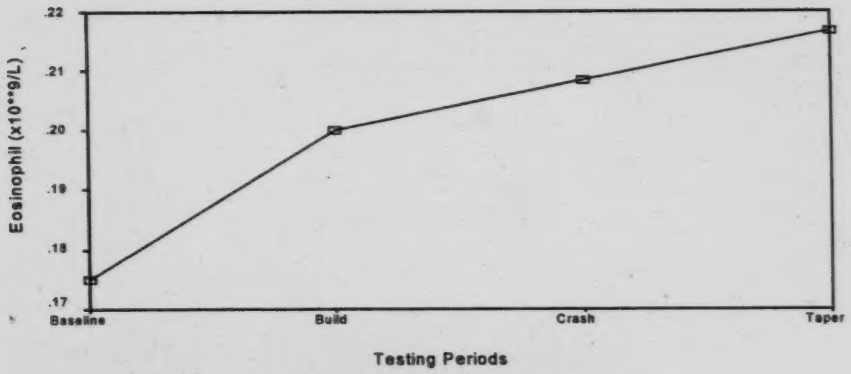

Figure 4.18 Eosinophil Concentration Across Testing Periods

2. T-cells and Natural Killer Cells:

Other immunological variables that were examined include the T-cells and natural killer (NK) cells (Table 4.10). As was previously mentioned, there are no results for the taper period due to an error in the lab during analysis.

Table 4.10 T-cells (CD4+ and CD8+) and Natural Killer Cells (CD16+CD56+)

MEASURE	Baseline (n=14)	Build (n=14)	Crash (n=14)
Total CD4+ (%)*	50[ab]	56[a]	59[b]
SE	2	1	2
Range	36-62	51-63	48-69
Total CD8+ (%)	28	28	27
SE	1	1	1
Range	13-33	24-32	20-34
CD4+: CD8+ Ratio*	1.9[a]	2.0[b]	2.3[ab]
SE	0.1	0.07	0.2
Range	1.3-2.9	1.7-2.6	1.5-3.4
CD16+CD56+ (%)	4.9	4.0	3.0
SE	1.1	0.8	0.9
Range	0.7-18.7	0.4-9.5	0.07-12.9

* = significant main effect of time at $p < 0.005$
matching alpha characters denote significance at $p < 0.05$ between test periods (based on *post hoc* analysis)

Repeated measures ANOVAs were run for each of the T-cells and natural killer cells. Results for the CD4+ cells showed a significant main effect of time, $F(1.536,19.962)=9.691$, $p=0.002$. *Post hoc* analysis revealed that the differences existed between the baseline and the build tests ($p=0.004$), and between the baseline and the crash tests ($p=0.005$) (Figure 4.19). Results for the CD8+ cells revealed no significant effect of time; nevertheless, a graph of the results is displayed below (Figure 4.20). In terms of the CD4+/CD8+ ratio, a significant main effect of time was also found, $F(2,26)=7.343$, $p=0.003$. *Post hoc* analysis determined that differences existed between the baseline test and the crash test ($p=0.006$), and also between the build test and the crash test ($p=0.044$) (Figure 4.21). For the natural killer cells (CD16+CD56+), the results from the repeated measures ANOVA found that there were no significant differences across time (Figure 4.22).

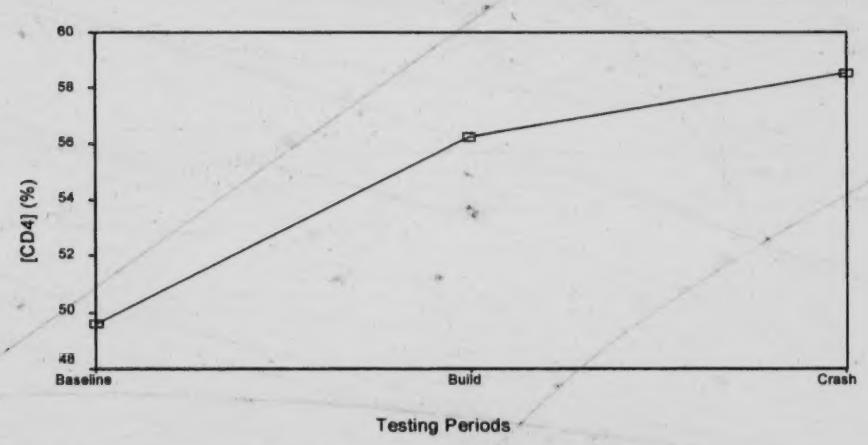

Figure 4.19 Percentage of Total CD4+ Cells Across Testing Periods

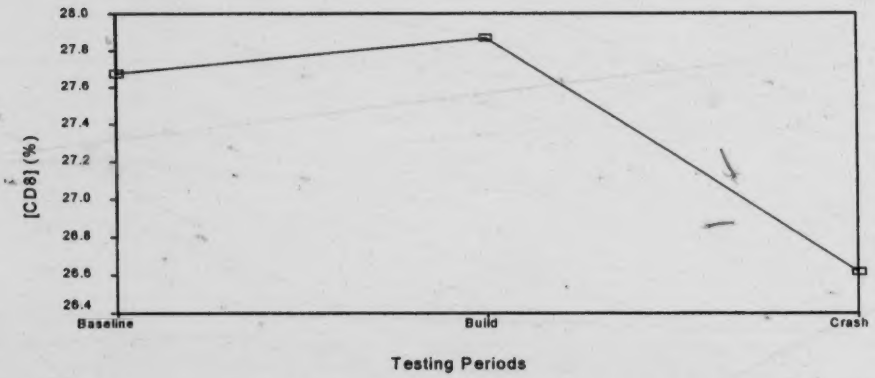

Figure 4.20 Percentage of Total CD8+ Cells Across Testing Periods

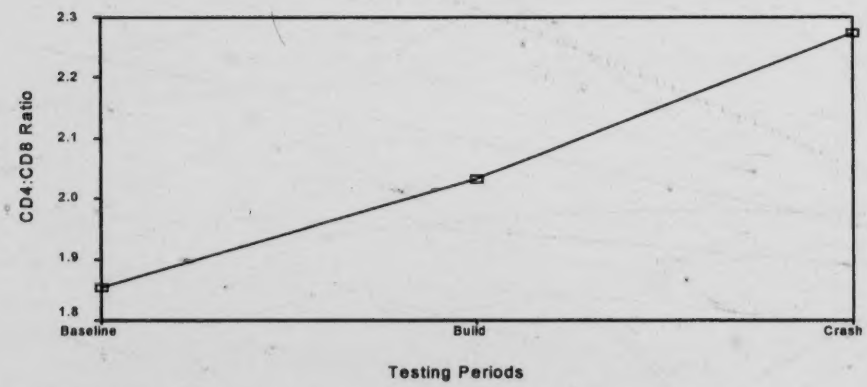

Figure 4.21 The CD4+/CD8+ Ratio Across Testing Periods

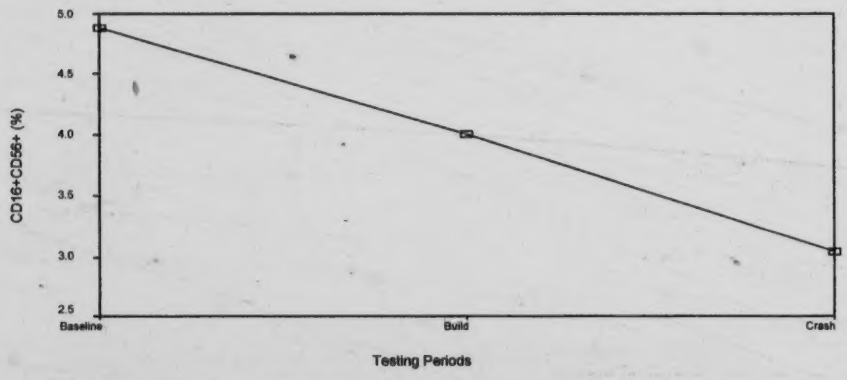

Figure 4.22 Percentage of CD16+CD56+Cells Across Testing Periods

3. Salivary Immunoglobin A:

Another immune function measure that was tested several times over the course of the study was S-IgA. The mean results at each test period can be observed in Table 4.11 and Figure 4.23. The repeated measures ANOVA for S-IgA revealed that there was no significant effect of time.

Table 4.11 Salivary Immunoglobin A Concentrations at Each Testing Session

MEASURE	Baseline (Sept. 9 & 12) n=14	Build (Oct. 15th) n=14	Crash (Nov. 12th) n=14	Taper (Nov. 25th) n=14
Salivary IgA (µg/ml)	93.8	158.8	128.1	118.0
SE	12.2	24.6	27.1	12.9
Range	43.3-229.0	50.4-337.9	33.0-434.5	52.1-195.5

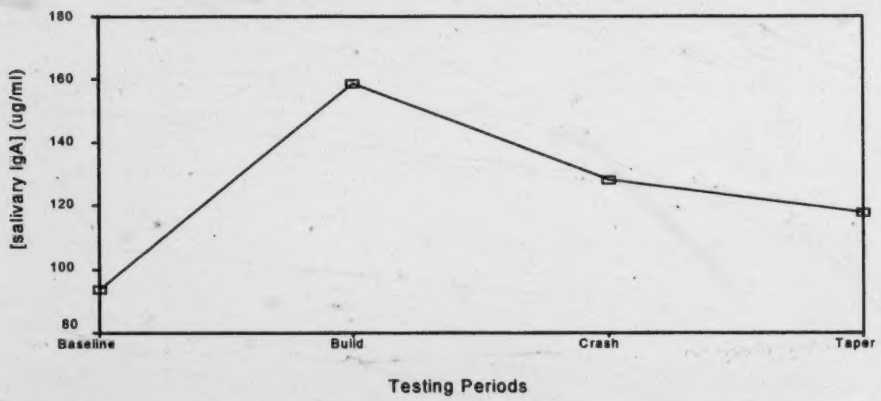

Figure 4.23 Salivary Immunoglobin A (S-IgA) Levels Across Testing Periods

a. *Salivary Immunoglobin A Assay Performance:*

Intra-assay performance was assessed for the S-IgA assay. Coefficients of variation (CV) were calculated for each set of duplicate wells, and the mean CV was determined for the test day. Seven known standards were assayed in order to make the standard curve. The mean CV for the standards was 4.5%. A total of 67 duplicate samples

were also run, which included the samples for the subjects for all the test days together. The resulting mean intra-assay CV for all of the samples was 3.825%.

4. Glutamine and Glutamate:

The final immunological measures that were examined were the amino acids glutamine and glutamate. Further the ratio between the two amino acids was calculated as glutamine: glutamate. The results for these variables can be observed below in Table 4.12.

Table 4.12 Glutamine and Glutamate Concentrations Across Time

MEASURE	Baseline (Sept. 9 & 12) n=14	Build (Oct. 15th) n=14	Crash (Nov. 12th) n=14	Taper (Nov. 25th) n=14
Glutamate (nmol/L)	79.4	68.7	64.5	76.9
SE	5.1	5.4	4.1	4.7
Range	55.1-127.0	42.5-108.5	45.6-92.8	58.7-110.7
Glutamine* (nmol/L)	942.1^{abc}	827.9^{ad}	783.5^{be}	683.4^{cde}
SE	42.2	42.2	23.1	20.2
Range	582.2-1182.7	600.2-1171.8	640.9-938.4	556.8-774.7
Glutamine: Glutamate*	12.2^{a}	12.7^{b}	12.8^{c}	9.2^{abc}
SE	0.7	0.8	0.9	0.5
Range	9.1-8.1	8.4-7.6	7.8-18.7	5.1-12.0

* = significant main effect of time at $p < 0.02$
matching alpha characters denote significance at $p < 0.05$ between test periods (based on *post hoc* analysis)

As was previously mentioned, the significance for glutamine, glutamate and the glutamine:glutamate ratio was set a $p < 0.02$, based on the Bonferroni correction factor (0.05/3). The results for the repeated measures ANOVA for glutamate revealed no significant effect of time. There was, however, a significant difference across time for glutamine, $F(3,39)=16.628$, $p=0.000$. *Post hoc* analysis determined that the differences occurred between the baseline and build tests ($p=0.024$), between the baseline and crash

tests (p=0.001), between the baseline and taper tests (p=0.000), between the build and taper tests (p=0.004), and also between the crash and taper tests (p=0.000). In other words, glutamine showed a steady decrease throughout the 4 test periods (Figure 4.24). The results for the repeated measures ANOVA for the glutamine:glutamate ratio also revealed a significant main effect of time, F(3,39)=8.463, p=0.000. *Post hoc* analysis determined that the differences existed between the baseline and taper tests (p=0.001), between the build and taper tests (p=0.003), and also between the crash and taper tests (p=0.000) (Figure 4.25).

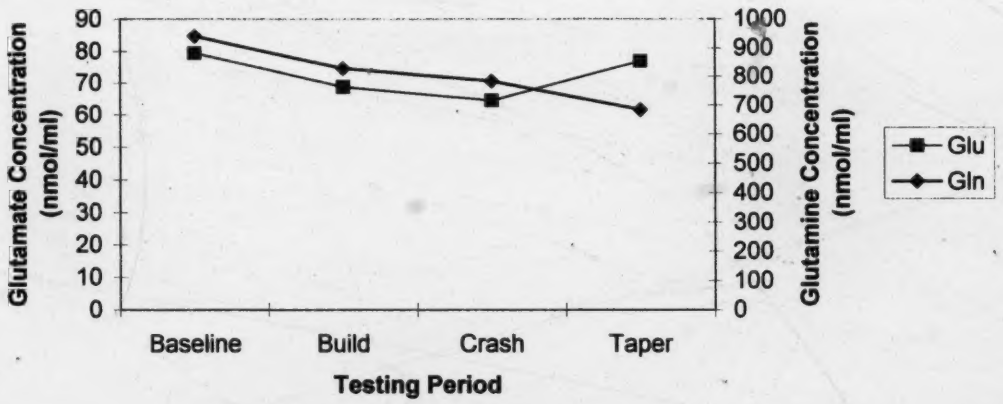

Figure 4.24 Comparison of Glutamate and Glutamine Concentrations Across Testing Periods

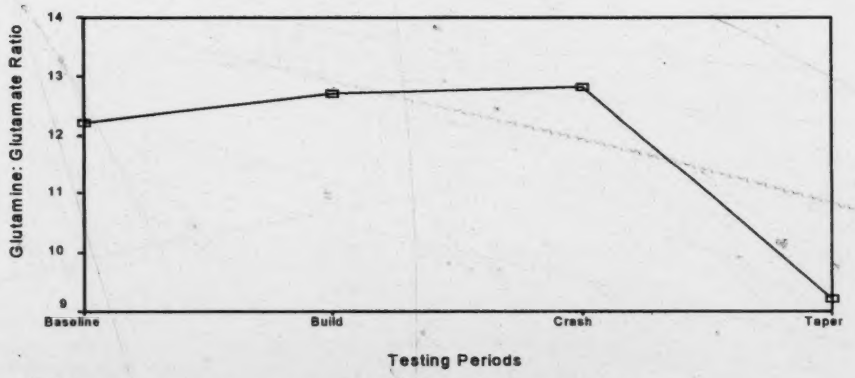

Figure 4.25 Glutamine: Glutamate Ratio Across Testing Periods

F) *HORMONAL MEASURE:*

1. Cortisol:

Cortisol concentrations were determined from saliva samples given by the subjects at each test period. An afternoon (4-5 PM) and an evening (8-10 PM) sample was requested of the subjects on the day prior to each of the main testing sessions. Unfortunately, several subjects forgot to provide their samples at the appropriate time of day, and therefore there were several missing samples. For the afternoon tests, only 6 subjects gave saliva samples at each of the testing sessions. For the evening tests, a total of 7 subjects provided a complete set of saliva samples. On the day of testing, the subjects also provided a morning (6-8AM) saliva sample. For the morning samples, a complete data set (n=14) was obtained. When looking at all of the afternoon, evening and morning samples, only 3 subjects provided a complete data set. Therefore, for presentation in this chapter, a decision was made to display the mean results of the 14 subjects who gave all of their morning saliva samples (Table 4.13), as well as the afternoon, evening, and morning results of the 3 subjects who completed all of their samples (Table 4.14).

Table 4.13 Morning Salivary Cortisol Concentrations for all 14 subjects

MEASURE (nmol/L)	Baseline	Build	Crash	Taper
Morning Cortisol (6-8AM)* n=14	35.7^{ab}	33.4^{c}	26.9^{ad}	18.6^{bcd}
SE	3.0	4.0	2.6	3.9
Range	18.7-54.9	12.7-69.7	13.0-48.1	0.0-54.1

* = significant main effect of time at $p < 0.05$
matching alpha characters denote significance at $p < 0.05$ between test periods (based on *post hoc* analysis)

For the morning samples of the 14 subjects, a repeated measures ANOVA was run. Results from the ANOVA demonstrated that there was a significant main effect of time,

$F(3,39)=6.962$, $p=0.001$. *Post hoc* analysis determined that the differences existed between the baseline and the crash tests ($p=0.021$), between the baseline and taper tests ($p=0.001$), between the build and the taper tests ($p=0.012$), and also between the crash and taper tests ($p=0.005$). That is, morning cortisol samples showed a steady decline across the testing periods (Figure 4.26).

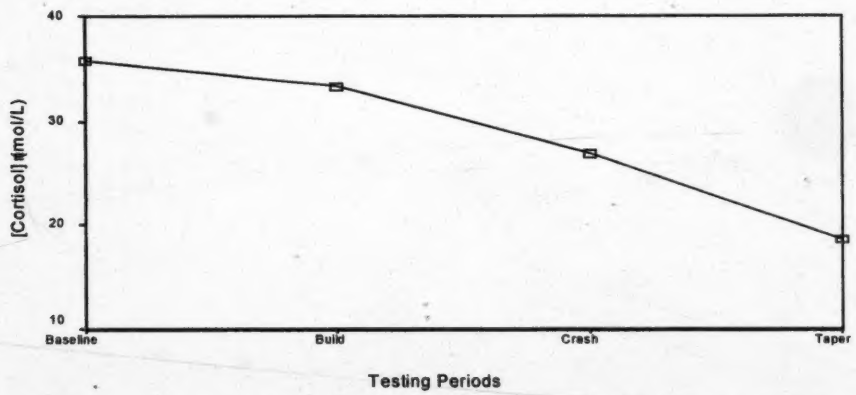

Figure 4.26 Morning Cortisol Concentration for all 14 Subjects at Each Test Period

Table 4.14. Afternoon, Evening and Morning Cortisol Concentrations for the 3 Subjects who Provided All of the Saliva Samples.

Cortisol Concentration (nmol/L)		SUBJECT #			
		03	11	14	Average
Afternoon (4-5 PM)	Baseline	10.0	12.3	15.6	12.6
	Build	8.8	0.7	33.8	14.4
	Crash	5.4	7.7	11.3	8.1
	Taper	0	7.8	3.6	3.8
Evening (8-10 PM)	Baseline	5.4	9.7	3.1	6.1
	Build	10.4	9.4	0	6.6
	Crash	0	7.2	0	2.4
	Taper	0	0	0	0
Morning (6-8AM)	Baseline	18.7	34.5	27.7	27.0
	Build	18.0	25.6	35.4	26.3
	Crash	18.2	21.1	37.8	25.7
	Taper	11.9	11.0	23.9	15.6

Note: the afternoon and evening samples were obtained the day prior to the main testing session where the morning sample was given.

In terms of the 3 subjects who provided a complete data set for cortisol, the results are simply descriptive in nature. This is due to the fact that a repeated measures ANOVA could not be run because of the small sample size. Therefore, a graphical representation of the mean results for the morning, afternoon, and evening cortisol concentrations across testing periods can be found in Figure 4.27. It is very clear from looking at the graph that the lowest cortisol values were observed during the taper period for all times of the day. Further, the results for the 3 subjects revealed that the morning cortisol samples were always of highest concentration (compared to the afternoon and evening samples). Figure 4.28 demonstrates how the cortisol levels changed based on the time of day of testing.

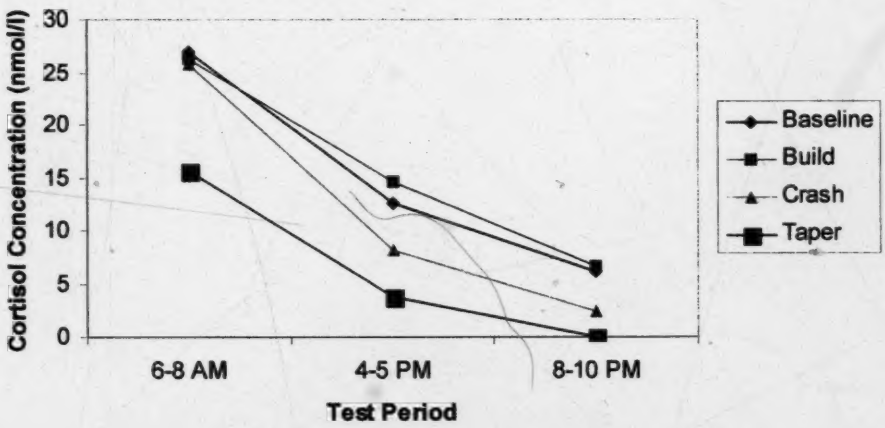

Figure 4.27 Morning, Afternoon and Evening Cortisol Concentrations for the 3 Subjects who Completed all of the Tests

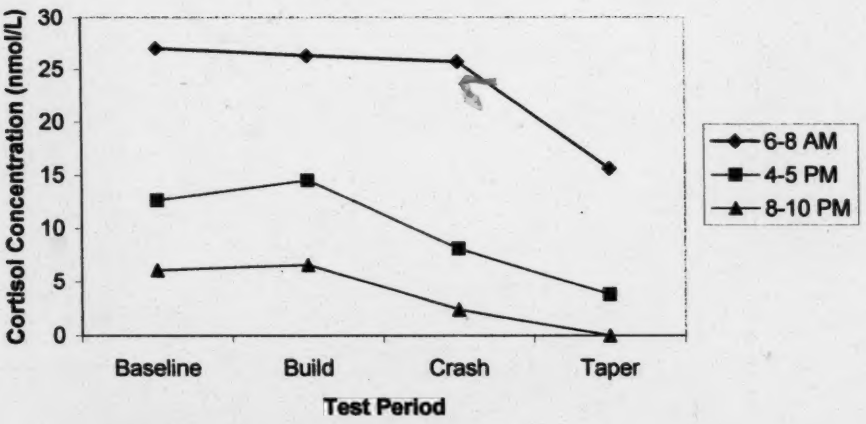

Figure 4.28 Cortisol Concentrations By Time of Day For Each Test Period for the 3 Subjects who Completed all of the Tests

a. Cortisol Assay Performance:

Inter- and intra- assay performance was assessed for the cortisol radioimmunoassay (RIA). All of the saliva samples were analyzed on a single lab day, however samples with a high coefficient of variation (CV) were repeated 3 days later with the same RIA. Coefficients of variation (CV) were calculated for each set of duplicate tubes, and then the mean CV was calculated for each lab day. For the first day of lab work, 2 researchers performed the analysis. Each researcher performed the assay for the same subjects for each of the test periods. One of the researchers obtained a mean CV of 3.4% for the standards, and a mean CV of 14.4% for the samples. The other researcher obtained a mean CV of 5.3% for the standards, and a mean CV of 26.1% for the samples. These coefficients of variation excluded the high CV samples that were repeated 3 days later. For the repeated assay, a mean CV of 7.3% was obtained for the standards, and a mean CV of 27.4% for the samples. Therefore, the mean of these 3 intra-assays is 5.3% for the standards, and 22.6% for the samples, which is the inter-assay CV for the cortisol RIA in this study.

G) *CARDIOVASCULAR MEASURES:*

Cardiovascular measures were also run at each of the morning testing sessions. In terms of the measures, the dynamic postural heart rate (Rusko) test was performed as well as spectral heart rate analysis (from the Omegawave Sport Technology system). These two tests provided 9 heart rate variability measures. Therefore, using the Bonferroni correction factor (0.05/9), significance was set at $p<0.006$.

Based on the results from the 10-minute dynamic postural heart rate (Rusko) test, two calculations were made. Firstly, the average heart rate was determined for the last 30 seconds of the test (while the subject was standing). Secondly, a mathematical formula was applied to calculate a Rusko "score" (Parent & Norris, 2001, unpublished). The formula utilized was as follows: (0.1*average HR of the last minute of lying) + (0.3*average HR of the last minute of standing).

Table 4.15 displays the mean results for both of these calculations across test periods.

Table 4.15 Results from the Dynamic Postural Heart Rate Test (Rusko)

MEASURE	Baseline (Sept. 9 & 12) n=14	Build (Oct. 15th) n=14	Crash (Nov. 12th) n=14	Taper (Nov. 25th) n=14
Average Heart Rate (bpm)	89.3	82.0	81.7	86.5
SD	12.6	13.0	11.9	16.4
Range	68.9-107.0	53.2-101.8	66.2-102.0	55.8-117.8
Calculated Rusko Score	32.57	30.32	30.07	31.24
SD	4.6	4.7	3.8	5.2
Range				

For both the calculated Rusko score and the average heart rate (for the last 30 seconds of standing), the repeated measures ANOVAs results could not be used due to violations in Mauchly's test of sphericity. As a result, multivariate tests were run for both

of these variables. However, results from the multivariate tests revealed that there was no significant effect of time. Figure 4.29 demonstrates that both variables followed the same trend across test periods.

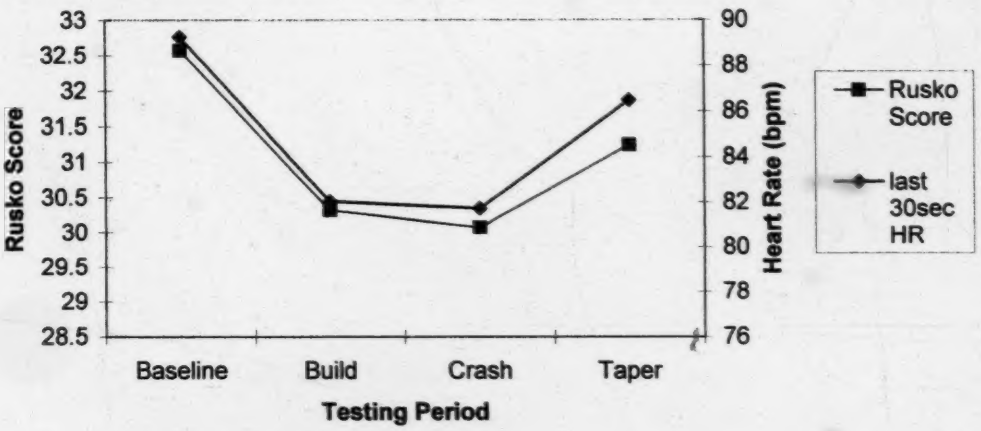

Figure 4.29 Comparison of Calculated Rusko Scores and the Average Heart Rate for the Last 30 Seconds of the Rusko Test

The results from the spectral heart rate analysis were used to make 6 calculations (Table 4.16). One calculation involved computing a mathematical score for total heart rate variability. This calculation was based on observation by Norris (2003, personal communication). Three of the other values obtained from the spectral heart rate analysis were the percentages of the very low frequency, low frequency and high frequency components of heart rate modulation. The last two values obtained from the spectral analysis were numerical values for vagus regulation mechanisms and for sympathetic regulation mechanisms.

Table 4.16 Heart Rate Variability Results (from the OmegaWave Sport Technology System)

MEASURE	Baseline (Sept. 9 & 12)	Build (Oct. 15th)	Crash (Nov. 12th)	Taper (Nov. 25th)
Total HRV Score n=14	15.6	15.0	16.0	16.0
SE	0.8	0.9	1.1	1.4
Range	11-20	10-24	10-22	12-27
VLF Spectrum (%) n=10	6.9	8.1	6.8	6.5
SE	1.3	2.4	2.3	1.2
Range	2.7-16.5	0.9-27.8	1.2-25.3	1.8-11.7
LF Spectrum (%) n=10	37.4	46.8	35.7	33.3
SE	6.3	6.1	5.7	4.9
Range	15.8-61.4	22.9-90.2	19.4-69.2	3.6-53.6
HF Spectrum (%) n=10	55.7	45.2	57.4	60.2
SE	6.2	5.4	6.4	5.2
Range	24.8-79.1	8.9-75.3	20.6-77.3	38.1-94.7
Vagus Regulation Mechanisms n=14	0.353	0.359	0.434	0.420
SE	0.02	0.03	0.03	0.03
Range	0.21-0.51	0.24-0.63	0.28-0.60	0.26-0.64
Sympathetic Regulation Mechanisms n=14	26.68	27.50	25.59	23.36
SE	1.4	2.3	1.9	1.5
Range	20.0-38.5	15.0-45.0	11.0-43.0	14.0-35.0

Note: HRV = heart rate variability, VLF = very low frequency, LF= low frequency, HF=high frequency

For each of the variables derived from the spectral heart rate analysis, repeated measures ANOVAs were run. For all of these variables, no significant main effect of time was observed. Graphical representations of the results can be observed in Figures 4.30, 4.31 and 4.32 below.

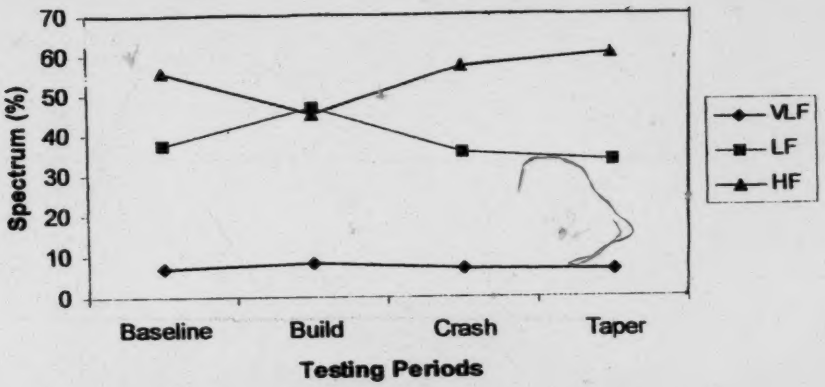

Figure 4.30 Very Low Frequency, Low Frequency and High Frequency Modulators of Heart Rate as determined from Spectral Analysis Across Testing Periods

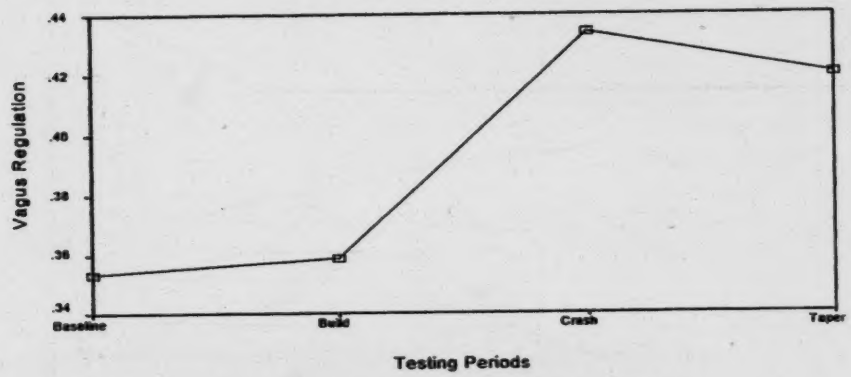

Figure 4.31 Vagus Regulation Mechanisms Across Testing Periods

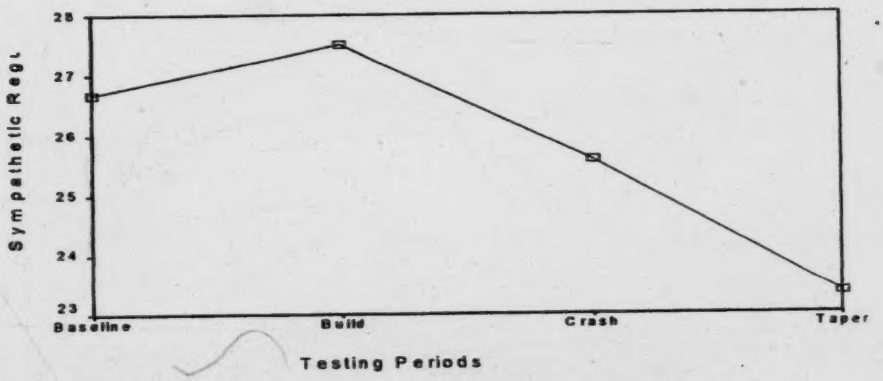

Figure 4.32 Sympathetic Regulation Mechanisms Across Testing Periods

H) PERFORMANCE MEASURES:

The last set of variables that was analyzed was swimming performance. The performance variables that were determined or calculated include the 100m time trial, a predicted 100m time (based on the results from the 4 x 50m test set), and a predicted 400m time (based on extrapolation of the heart rate velocity graph for the 5 x 200m test set). Therefore, there were three swimming test results. Based on this, the Bonferroni correction factor was applied (0.05/3), and significance was set *a priori* at $p<0.02$. Examination of the slope of the heart rate velocity graph for the 5 x 200m test sets was also done to provide insight into the heart rate response to changes in swimming velocity. Further, the results from the major competition (the Canadian National Championships or the Prairie Winter Invitational Swim Meet) were analyzed to ultimately test how effective the training season was for each of the subjects.

1. 100m Time Trial and the 4 x 50m Test Set (Predicted 100m Time):

Mean results for the 100m time trials and the predicted 100m times can be found in Table 4.17. As a reminder, swimmers were instructed to swim their best stroke for both the 100m time trial and the 4 x 50m test set. Unfortunately, 3 swimmers did not complete the tests at all of the test periods. Therefore, only the results from 11 of the subjects are displayed below (4 swam butterfly, 1 swam backstroke, 1 swam breaststroke, and 5 swam freestyle).

Table 4.17 Results for the 100m Time Trial and the Predicted 100m (calculated from the 4 x 50m test set)

MEASURE	Baseline (Sept. 12th) n=11	Build (Oct. 15th) n=11	Crash (Nov. 12th) n=11	Taper (Nov. 23rd) n=11
Predicted 100m*	1:01.3[abc]	1:00.4[ad]	59.4[bd]	59.9[c]
SE	1.5	1.6	1.5	1.8
Range	53.3-1:09.0	52.3-1:10.7	51.2-1:09.7	51.6-1:12.2
100m Time Trial*	1:04.1[abc]	1:02.7[ad]	1:01.9[bd]	1:02.2[c]
SE	1.9	1.8	1.8	1.7
Range	54.2-1:11.2	53.3-1:13.1	53.5-1:12.1	52.6-1:13.4
Difference between predicted 100m and time trial (seconds)	2.8	2.3	2.5	2.3

* = significant main effect of time at $p < 0.02$
matching alpha characters denote significance at $p < 0.05$ between test periods (based on *post hoc* analysis)

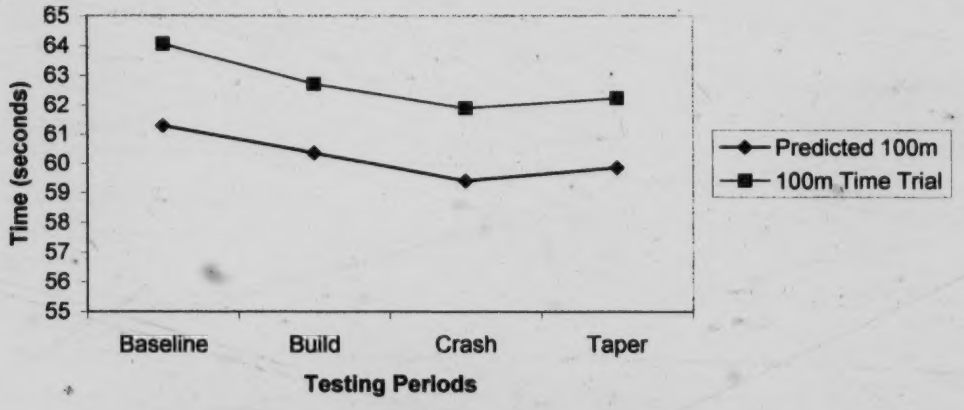

Figure 4.33 Comparison between the 100m Time Trial Results and the Predicted 100m Time (based on the 4 x 50m Test Set) Across Testing Periods

The repeated measures ANOVA for the predicted 100m times determined that there was a significant main effect of time, $F(3,30)=7.1$, $p=0.001$. *Post hoc* analysis revealed that the differences existed between the baseline and build tests ($p=0.023$), between the baseline and crash tests ($p=0.002$), between the baseline and taper tests ($p=0.044$), and between the build and crash tests ($p=0.008$). The repeated measures ANOVA for the 100m time trial found very similar results. The ANOVA revealed that

there was a significant main effect of time. *Post hoc* analysis determined that the differences existed between the baseline and build tests (p=0.025), between the baseline and crash tests (p=0.008), between the baseline and taper tests (p=0.015), and between the build and crash tests (p=0.034). Figure 4.33 displays the results for the predicted 100m times as well as for the 100m time trial.

2. 5 x 200m Test Set:

The mean results for the 5 x 200m test set can be seen in Table 4.18. Specifically, the predicted 400m time is given along with the slope and intercept of the heart rate/ velocity graph. For the 5 x 200m test, 12 of the subjects completed the test at each of the sessions (11 of the athletes swam freestyle and only one subject swam backstroke at each of the test sessions). The results from the other 2 subjects involved in the study were removed because they were too ill to complete the test during the taper period.

Based on the repeated measures ANOVA for the slope, it was determined that there was no significant effect of time for this variable. In terms of the results for the predicted 400m time, the repeated measures ANOVA could not be used due to a violation in Mauchly's test of sphericity. Therefore, a multivariate test was run which determined that significance was p=0.001. *Post hoc* analysis using the Scheffe test revealed that no significant differences existed between any of the time points. Figure 4.34 illustrates the results for the predicted 400m time, and Figure 4.35 displays the results for the slope of the 5 x 200m test set.

Table 4.18 Results from the 5 x 200m test set

MEASURE	Baseline (Sept. 12th) n=12	Build (Oct. 15th) n=12	Crash (Nov. 12th) n=12	Taper (Nov. 23rd) n=12
Slope of the HR/velocity graph	269.2	260.9	202.7	230.2
SE	50.9	40.9	15.2	49.8
Range	92.7-684.7	97.9-566.7	130.9-276.9	110.4-746.8
Intercept	-171.1	-181.7	-103.7	-140.3
SE	64.1	54.7	17.7	66.2
Range	-694.1-34.7	-590.0-17	-198.2-1.8	-823.9-24.9
r^2	0.96	0.94	0.96	0.95
SE	0.007	0.02	0.007	0.01
Range	0.93-0.99	0.77-0.99	0.92-0.99	0.84-0.99
Predicted 100m split at Hr_{max} (seconds)	70.4	66.4	66.1	66.1
SE	1.9	1.8	1.4	1.5
Range	56.1-75.9	53.5-75.8	60.5-76.7	57.9-76.2
Predicted 400m Time (seconds)	281.4	265.7	264.6	264.4
SE	7.7	7.2	5.5	6.1
Range	224.4-303.6	214.0-303.2	242.0-306.8	231.6-304.8

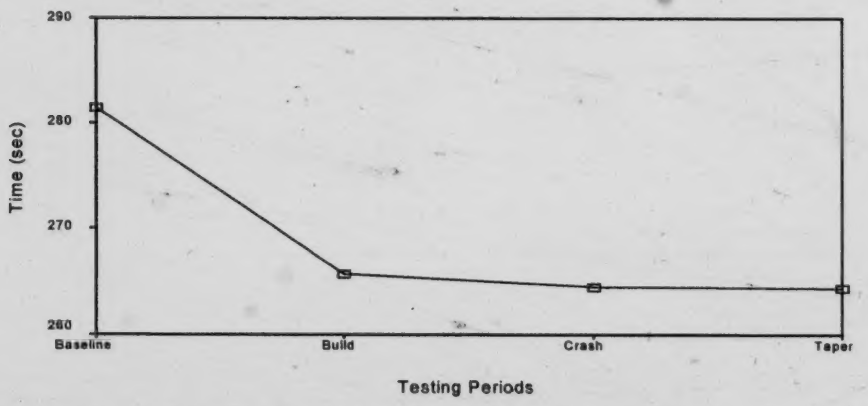

Figure 4.34 Predicted 400m Swimming Times Across Testing Periods

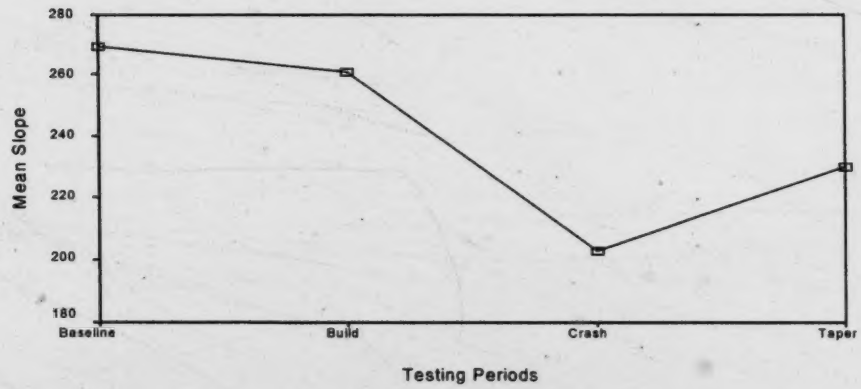

Figure 4.35 Mean Slope of the 5 x 200m Test Set Across Testing Periods

3. Competition:

The final and most substantial variable examined in this study was the performance of the subjects at their major competition (Canadian National Championships or Prairie Winter Invitational). Based on the results from the competition, a percentage of best time was determined for each athlete for their 3 main events. Table 4.19 displays these percentages as well as the average percentage of best time for each subject. The final column in the Table provides an indication as to how well each subject performed at the major competition.

Table 4.19 Each Subject's Performance at the Major Competition in their Best Three Events

Subject	Event # 1	% of Best Time	Event # 2	% of Best Time	Event # 3	% of Best Time	Average % Best of all 3
01	400 FR	92.7	800 FR	94.6	NA	NA	93.7
02	400 FR	101.2	800 FR	99.7	200 FR	99.4	100.1
03	100 FR	98.0	200 FR	97.9	400 FR	98.5	98.1
04	200 FR	99.8	100 FLY	97.8	400 FR	101.9	99.8
05	200 IM	97.9	400 IM	97.2	NA	NA	97.6
06	100 BR	98.5	200 BR	98.9	50 BR	100.7	99.3
07	400 FR	99.9	200 BK	100.0	200 FR	99.9	99.9
08	100 FR	99.4	200 FR	100.6	50 FR	95.8	98.6
09	50 BK	101.4	100 BK	101.3	200 BK	98.3	100.3
10	200 BK	101.8	200 FR	100.1	100 BK	100.8	100.9
11	200 BR	98.4	100 BR	95.5	50 BR	96.8	96.9
12	1500 FR	100.7	200 FLY	98.0	400 FR	98.5	99.1
13	200 FLY	99.2	100 FLY	97.4	50 FLY	100.3	99.0
14	400 FR	96.1	400 IM	96.8	800 FR	97.9	96.9

Legend: FLY= Butterfly; BK= Backstroke; BR= Breaststroke; FR= Freestyle; IM= Individual Medley (all four strokes)

GROUP RESULTS: RESPONDERS VS. NON-RESPONDERS

Based on the results from the major competition, the athletes were separated into three groups. Athletes were placed into group one (RESPONDERS) if they achieved 100% or greater of their personal best times as an average of their performance in their best 3 events at their major competition. Subjects were placed into group two if they received an average of 97% -99%, and into group 3 (NON-RESPONDERS) if they achieved an average of less than 97%. According to Norris (2003, personal communication), competitive swimmers should be able to achieve 97% -98% of their personal best times at in-season (non-tapered) competitions. Therefore, it is logical that at a major competition (which involves a taper), athletes who are sufficiently prepared should easily perform better than 97% of their best times. This provided the rationale for establishing the non-responders as those who

achieved less than 97%. Table 4.19, which is displayed above, provides the specific average that each athlete obtained at the major competition for their best three events. Table 4.20 shows the subjects that were placed into each group, and also which athletes were designated as responders or non-responders. It was coincidental that there were 3 athletes who fell into each of the two classifications.

Table 4.20 Designation /Classification for Subjects Based on Performance at Competition

Subject Number	Group	Classification
01	3	Non-responder
02	1	Responder
03	2	
04	2	
05	2	
06	2	
07	2	
08	2	
09	1	Responder
10	1	Responder
11	3	Non-responder
12	2	
13	2	
14	3	Non-responder

The following section examines the results for the responders and the non-responders for each of the variables. As there are only 3 subjects in each of these categories, descriptive statistics and graphs are used to illustrate the results. Only the responders and non-responders are examined because it was deemed important to look at the extremes and to try and differentiate why the responders performed well (100% + best times), and the non-responders performed poorly (<97% best times).

A) *SUBJECT CHARACTERISTICS: RESPONDERS VS. NON-RESPONDERS:*

The three responders (R) and three non-responders (NR) were all females. This was fortunate as differences due to gender could be eliminated. The 3 responders were 16, 18 and 18 years of age throughout the study, and the 3 non-responders were 16, 16 and 18 years of age during the course of the study. In terms of anthropometric measurements, at the start of the study, the responders were taller, weighed less, had a smaller waist circumference, and had a lower body mass index (BMI) score compared to the non-responders (Table 4.21).

Table 4.21 Anthropometric Measures for Responders (R) Vs. Non-Responders (NR)

Measure	**Baseline** (Sept. 9 & 12)		**Build** (Oct. 15th)		**Crash** (Nov. 12th)		**Taper** (Nov. 25th)	
	R n=3	NR n=3	R n=3	NR n=3	R n=3	NR n=3	R n=3	NR n=3
Height (cm)	178.3	174.9	177.6	174.4	177.7	174.5	177.5	174.5
SE	2.2	0.8	2.5	0.6	2.3	0.8	2.4	0.8
Weight (Kg)	61.6	63.9	62.4	63.5	62.0	64.0	61.6	63.2
SE	1.9	2.3	2.1	3.0	2.2	2.8	2.0	3.2
Waist (cm)	69.3	70.8	70.7	71.2	70.3	70.7	70.0	70.3
SE	3.0	3.3	2.5	3.5	2.6	4.0	2.1	4.4
BMI (Kg/m^2)	19.4	20.9	19.8	20.9	19.6	21.0	19.6	20.8
SE	0.2	0.9	0.09	1.1	0.2	1.1	0.2	1.2

B) *ENERGY INTAKE: RESPONDERS VS. NON-RESPONDERS:*

In terms of the 3-day dietary records, the non-responders consumed more calories than the responders the first time they were tested (October 10-12th), but consumed less calories than the responders the second time they were tested (November 7-9th). A similar trend occurred for grams of protein and also for grams of fat consumed. Carbohydrate intake, on the other hand, was higher for the non-responders at both tests compared to the

responders. Further, it is important to note that the non-responders demonstrated a decrease in total calories from test one to test two, while the responders took in relatively the same amount of calories over the same time period (Table 4.22).

Table 4.22 Results from the 3-Day Dietary Records for Responders and Non-Responders

MEASURE	BUILD (October 10-12th)		CRASH (November 7-9th)	
	R	NR	R	NR
Calories (Kcal)	2751	3241	2740	2436
SE	244	516	76	123
Protein (g)	94	112	81	76
SE	14	26	15	10
Carbohydrate (g)	406	491	363	371
SE	4	92	14	5
Fat (g)	87	94	109	75
SE	23	5	16	6
Protein (%)	13	13	12	12
SE	1	1	2	1
Carbohydrate (%)	59	59	52	60
SE	5	2	4	2
Fat (%)	27	27	35	27
SE	4	2	4	1

C) *TRAINING: RESPONDERS VS. NON-RESPONDERS:*

All 6 of the responders and non-responders were chronically trained swimmers. Of the responders, all 3 of the athletes trained predominantly as middle-distance swimmers, however one of the athlete also trained frequently as a distance swimmer. For the non-responders, two of the subjects were distance swimmers and one was a middle-distance swimmer. A comparison of the weekly swim volume for the responders and non-responders can be seen in Table 4.23 and Figure 4.36. In general, it appears as though the non-responders swam more Km/week, especially during weeks 3, 5 and over the last 4 weeks of the study. Furthermore, a comparison of the time that each group spent doing dryland

activities can also be found in Table 4.23 and Figure 4.37. It appears, that the non-responders completed more dryland activities over the last 4 weeks of the study.

Table 4.23 Weekly Training Record for Responders and Non-Responders

TRAINING DATES	Swim Volume (Km)		Dryland time (min.)	
	R	NR	R	NR
Week 1: Sept. 16 - 22	11.5	11.0	20.0	80.0
Week 2: Sept. 23 - 29	17.4	17.5	68.3	51.7
Week 3: Sept. 30 - Oct. 6	20.6	29.0	23.3	38.0
Week 4: Oct. 7 - 13	36.8	38.1	103.3	34.4
Week 5: Oct. 14 - 20	32.1	39.7	31.7	14.2
Week 6: Oct. 21 - 27	28.5	30.6	26.7	22.0
Week 7: Oct. 28 - Nov. 3	30.0	56.1	28.3	49.5
Week 8: Nov. 4 - 10	41.7	48.7	53.3	37.6
Week 9: Nov. 11 - 17	35.5	43.5	28.3	52.9
Week 10: Nov. 18 - 24	30.9	33.7	36.7	48.5

Reminder: baseline test occurred before the start of training, the build test took place after week 4 (Oct.15th), the crash test occurred after week 8 (Nov.12th), and the taper test happened after week 10 (Nov.25th).

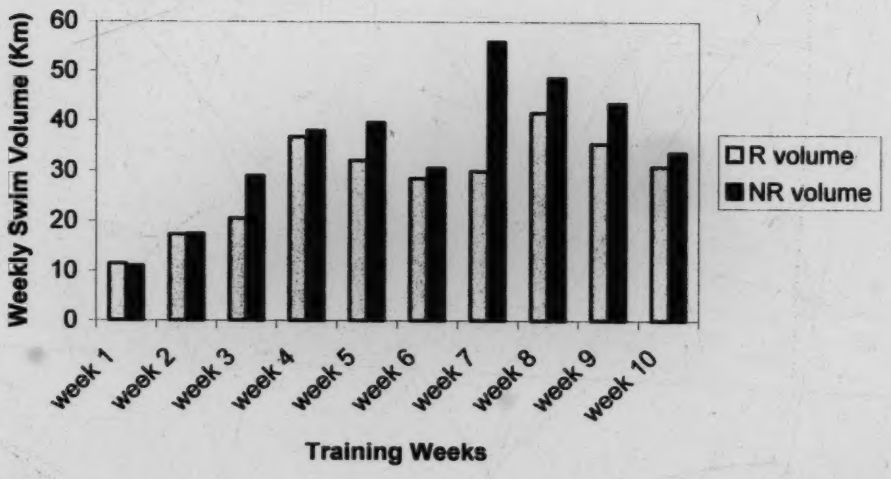

Figure 4.36 Comparison of Weekly Swim Volume for Responders and Non-Responders

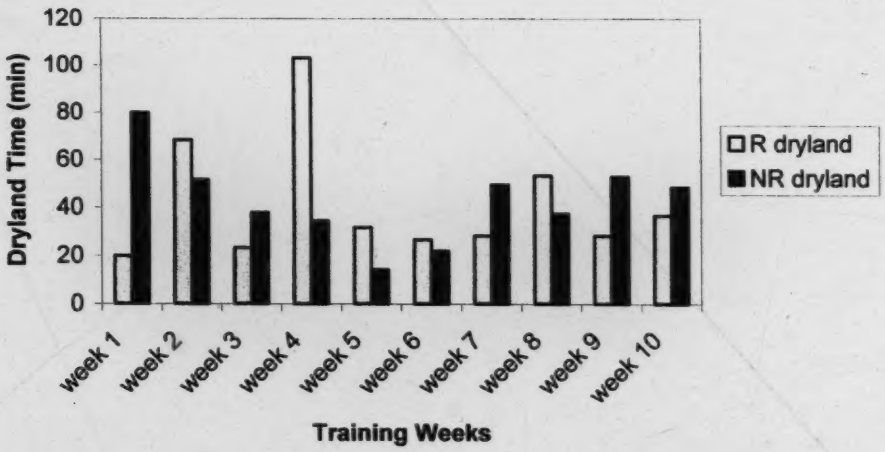

Figure 4.37 Time Spent Doing Dryland Activities / Week for Responders and Non-Responders

With reference to the subjective ratings of intensity, it appears as though the responders engaged in more intense training than did the non-responders. This can be seen from Figure 4.38. However, in terms of the subjective ratings of fatigue, it looks as though the non-responders had higher ratings of fatigue especially during the last 4 weeks of the study (Table 4.24 and Figure 4.39).

Table 4.24 Comparison of Subjective Ratings of Training Intensity and Fatigue between the Responders and Non-Responders

TRAINING DATES	Training Intensity		Fatigue	
	R	NR	R	NR
Week 1: Sept. 16 - 22	3.3	2.2	3.5	2.6
Week 2: Sept. 23 - 29	4.5	3.8	4.1	3.8
Week 3: Sept. 30 - Oct. 6	3.9	2.9	4.1	3.4
Week 4: Oct. 7 - 13	4.1	3.6	4.1	4.2
Week 5: Oct. 14 - 20	4.5	4.5	4.1	5.0
Week 6: Oct. 21 - 27	3.9	3.5	4.0	3.6
Week 7: Oct. 28 - Nov. 3	4.0	4.2	4.0	4.8
Week 8: Nov. 4 - 10	4.3	4.7	4.0	5.0
Week 9: Nov. 11 - 17	4.7	4.2	4.3	4.4
Week 10: Nov. 18 - 24	4.5	3.2	3.9	5.3

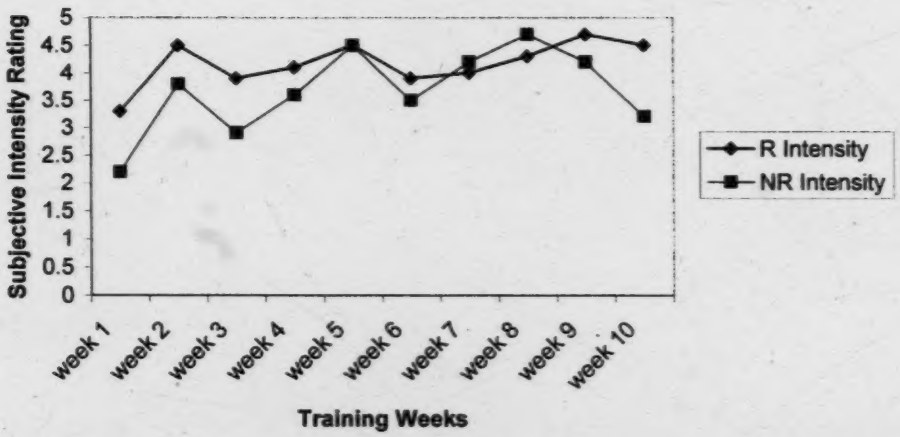

Figure 4.38 Weekly Subjective Ratings of Training Intensity for Responders and Non-Responders

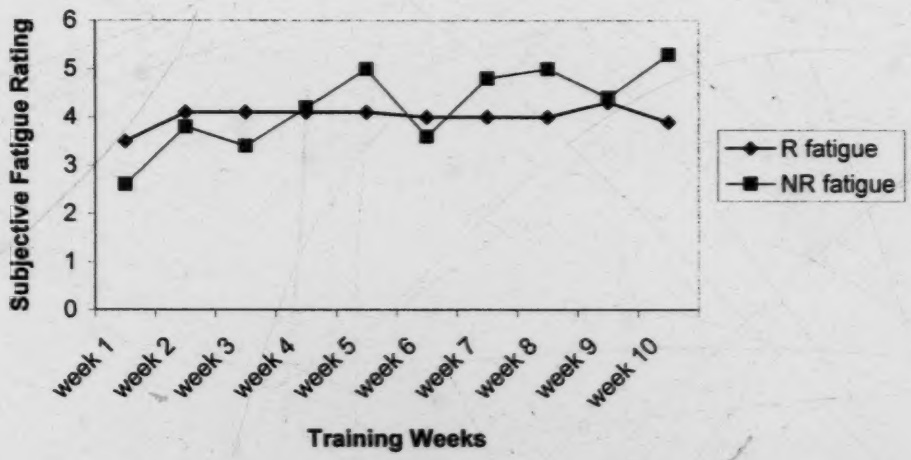

Figure 4.39 Weekly Subjective Ratings of Fatigue for Responders and Non-Responders

In the training log books, the responders and non-responders also recorded information on their frequency of illnesses and occurrence of menstruation. In terms of illnesses, the responders reported that they felt ill at 13.7% of the workouts they attended, and the non-responders stated that they were ill at 22.3% of the practices. In

relation to menstruation, the responders reported that they were menstruating for a mean of 15.2% of the days during the study, compared to the non-responders who reported that they had their period on 13.2% of the days. Further, responders suggested that the mean length of their period was 5.2 days, whereas the non-responders reported an average period of 4.5 days.

D) HEMATOLOGICAL MEASURES: RESPONDERS VS. NON-RESPONDERS:

Based on the results, it can be noted that the Hb levels were lower at each testing point for the non-responders compared to the responders. Generally, the RBC count, Hct and MCHC were lower at each test for the non-responders. As well, MCV was higher at each test for the non-responders (Table 4.25).

Table 4.25 Results for Tests Related to Red Blood Cells for Responders and Non-Responders

Measure	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)		Female reference range *
	R	NR	R	NR	R	NR	R	NR	
Hemoglobin (g/L)	142.0	136.8	144.0	131.0	145.7	133.7	144.7	140.3	120-160
Red blood cell count ($*10^{12}$/L)	4.7	4.5	4.7	4.2	4.8	4.3	4.8	4.5	4.1-5.1
Hematocrit (L/L)	0.41	0.41	0.42	0.39	0.42	0.39	0.42	0.41	0.36-0.46
Mean Cell Volume (fL)	87.3	89.8	88.3	91.0	88.3	90.3	88.3	90.7	78-100
MCHC (g/L)	344.0	339.3	345.3	340.3	346.3	345.0	342.0	342.3	310-360

* normal reference range provided by the Dynacare Kasper Medical Laboratory, Edmonton (2002)

Results for iron and its associated variables including TIBC and ferritin for each of the groups can be found in Table 4.26. Figure 4.40 demonstrates that the iron levels for the

non-responders were significantly lower at the crash and taper periods compared to the responders. Interestingly, two of the non-responders experienced iron levels that were below the normal female reference range during the crash period, and one of these girls also recorded very low iron levels during the taper period. On the other hand, one of the responders experienced very high iron levels (i.e. above the reference range) during the baseline, crash and taper tests. Figure 4.41 shows that TIBC started off at relatively similar concentrations for both the responders and non-responders, but then was lower at the build, crash and taper periods for the non-responders. In terms of ferritin, Figure 4.42 shows that the levels were much lower at baseline for the non-responders, but then became very comparable to the responders at the crash and taper periods.

Table 4.26 Comparison of Tests of Iron Status for Responders and Non-Responders

Measure	**Baseline** (Sept. 9 & 12)		**Build** (Oct. 15th)		**Crash** (Nov. 12th)		**Taper** (Nov. 25th)		**Female reference Range** *
	R	NR	R	NR	R	NR	R	NR	
Iron (umol/L)	16.8	**18.0**	11.7	**14.3**	16.3	**8.3**	21.3	**13.0**	8-25
TIBC (umol/L)	57.7	**58.2**	56.3	**52.0**	63.0	**54.3**	65.7	**56.3**	40-80
Ferritin (ug/L)	60.5	**39.8**	35.7	**26.0**	29.3	**26.7**	31.3	**29.7**	12-300

* normal reference range provided by the Dynacare Kasper Medical Laboratory, Edmonton (2002)

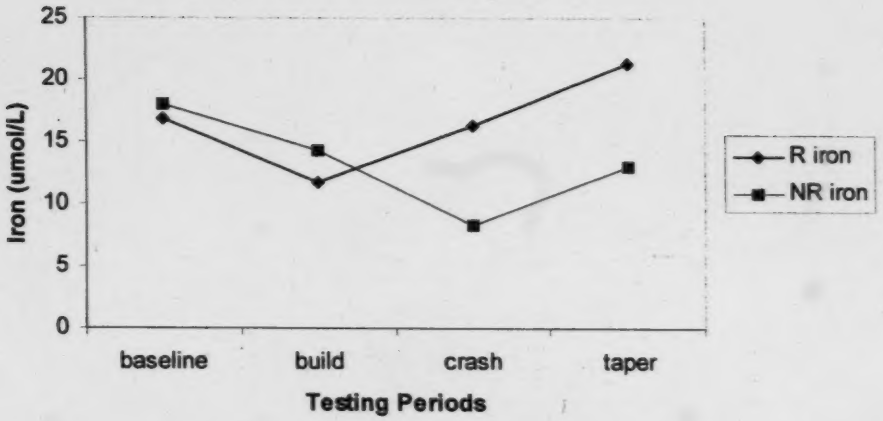

Figure 4.40 Iron Levels For Responders and Non-Responders Across Testing Periods

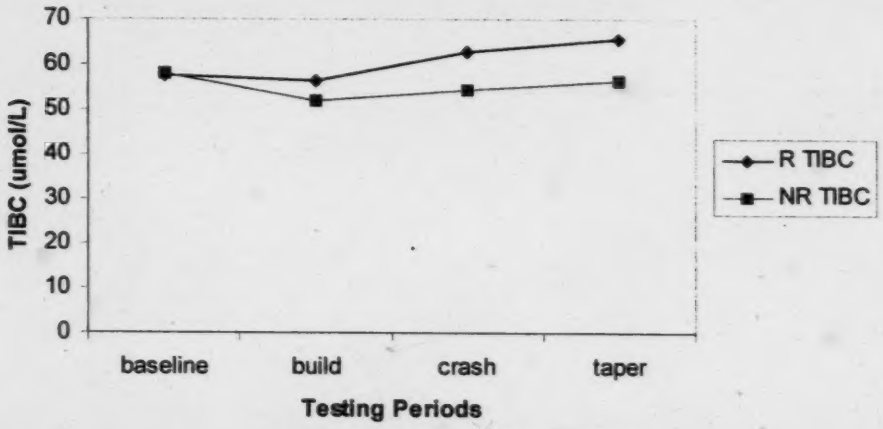

Figure 4.41 Total Iron Binding Capacity (TIBC) for Responders and Non-Responders Across Testing Periods

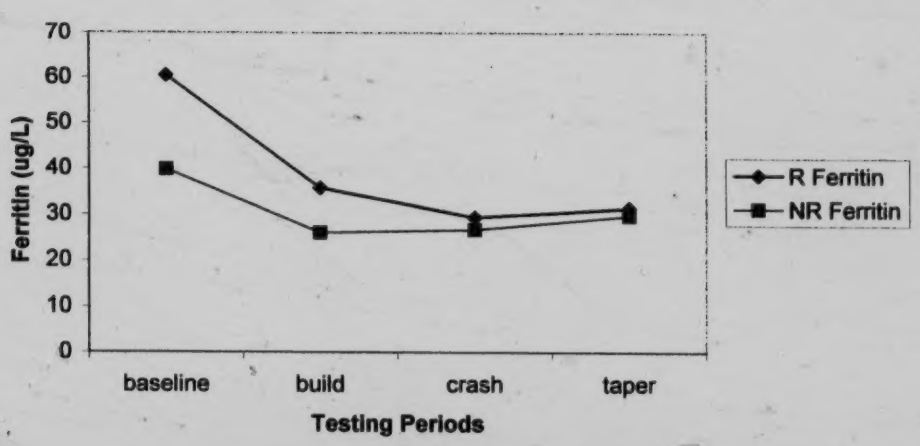

Figure 4.42 Ferritin Levels for Responders and Non-Responders Across Testing Periods

E) IMMUNOLOGICAL MEASURES: RESPONDERS VS. NON-RESPONDERS:

1. White Blood Cells and Differential:

Results for the white blood cells (WBC) and differential for the responders and non-responders illustrate that the WBC were at their highest level during the crash period and then at their lowest level during the taper period for the non-responders. For the responders, the WBC count remained fairly stable across the 4 test periods (Table 4.27, Figure 4.43). Further, graphs for each of the differential variables can be seen below (Figures 4.44 -4.48). While none of the WBC and differential results fell outside of the normal female reference range, it is interesting to note that many of the values increased during the crash period for the non-responders (except for lymphocytes and basophils).

Table 4.27 Results for White Blood Cells and Differential for Responders and Non-Responders

Measure	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)		Female Reference Range *
	R	NR	R	NR	R	NR	R	NR	
White blood cell ($x10^9$/L)	6.5	6.1	6.5	6.1	6.1	7.3	6.2	5.7	4.5-13.0
Neutrophil ($x10^9$/L)	4.1	3.0	2.9	2.9	2.8	3.7	3.0	2.9	1.8-8.0
Lymphocyte ($x10^9$/L)	2.0	2.5	2.8	2.4	2.6	2.2	2.5	1.8	1.2-5.2
Monocyte ($x10^9$/L)	0.5	0.5	0.5	0.5	0.5	1.0	0.4	0.5	0.0-1.0
Eosonophil ($x10^9$/L)	0.13	0.13	0.17	0.17	0.13	0.27	0.13	0.15	0.0-0.7
Basophil ($x10^9$/L)	0.0	0.03	0.07	0.1	0.07	0.07	0.13	0.15	0.0-0.3

* normal reference range provided by the Dynacare Kasper Medical Laboratory, Edmonton (2002)

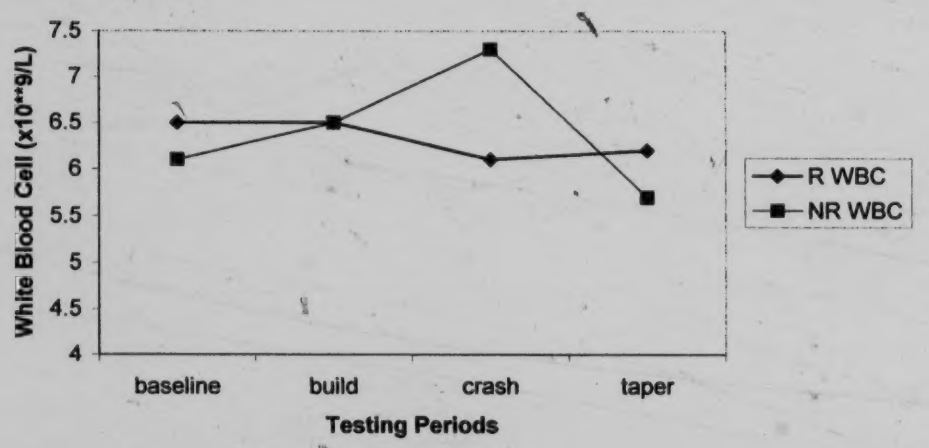

Figure 4.43 White Blood Cell Count for Responders and Non-Responders Across Testing Periods

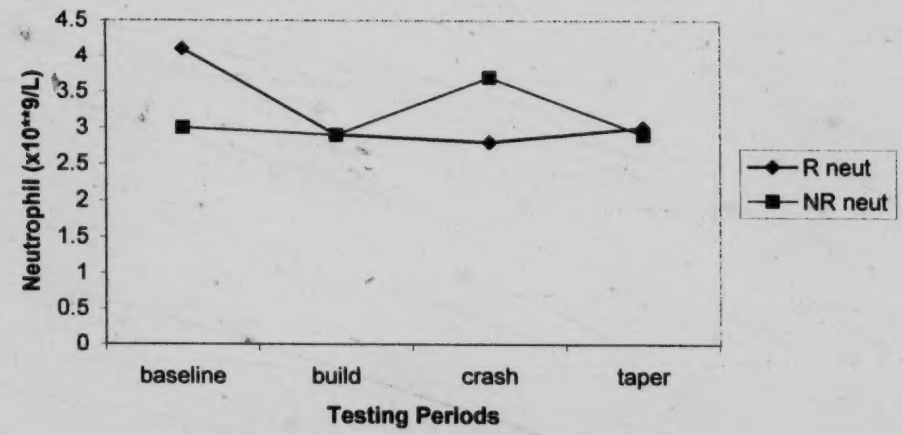

Figure 4.44 Neutrophil Concentration for Responders and Non-Responders Across Testing Periods

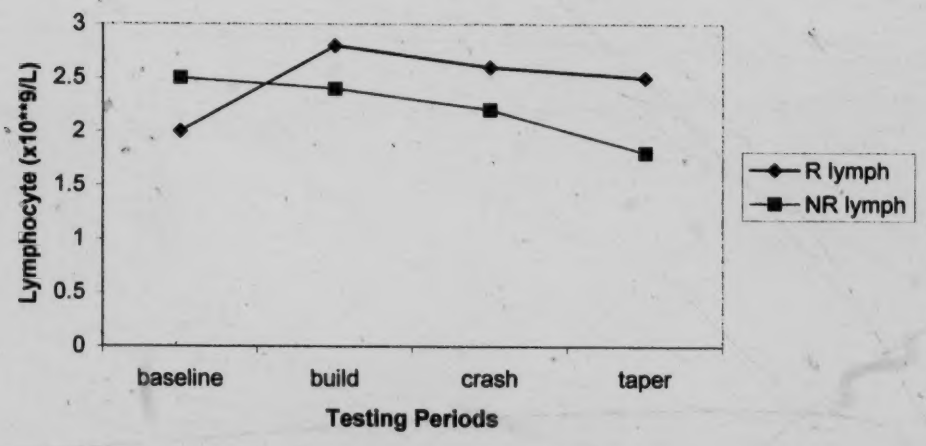

Figure 4.45 Lymphocyte Concentration for Responders and Non-Responders Across Testing Periods

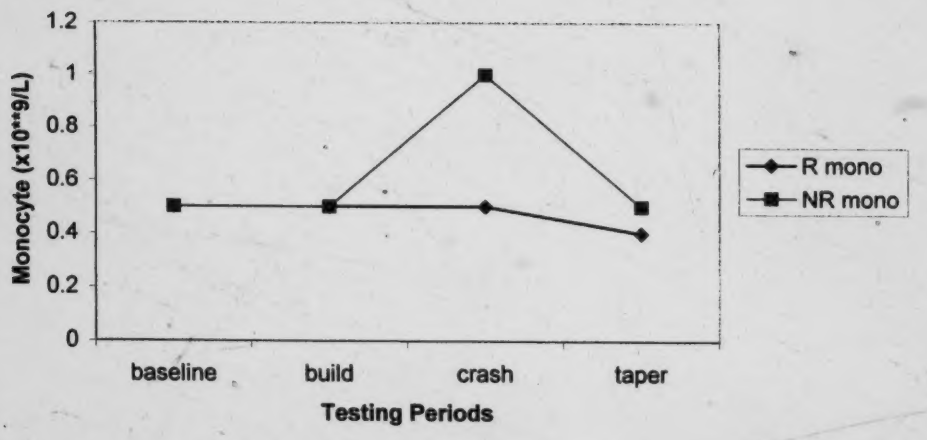

Figure 4.46 Monocyte Concentration for Responders and Non-Responders Across Testing Periods

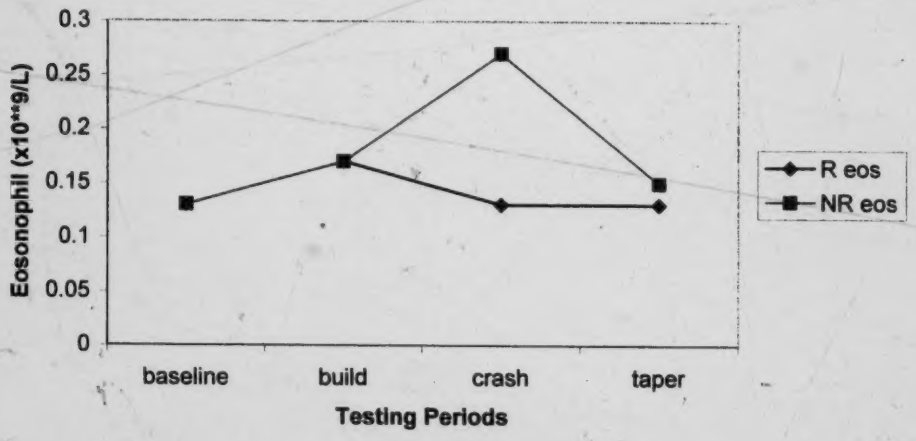

Figure 4.47 Eosonophil Concentration for Responders and Non-Responders Across Testing Periods

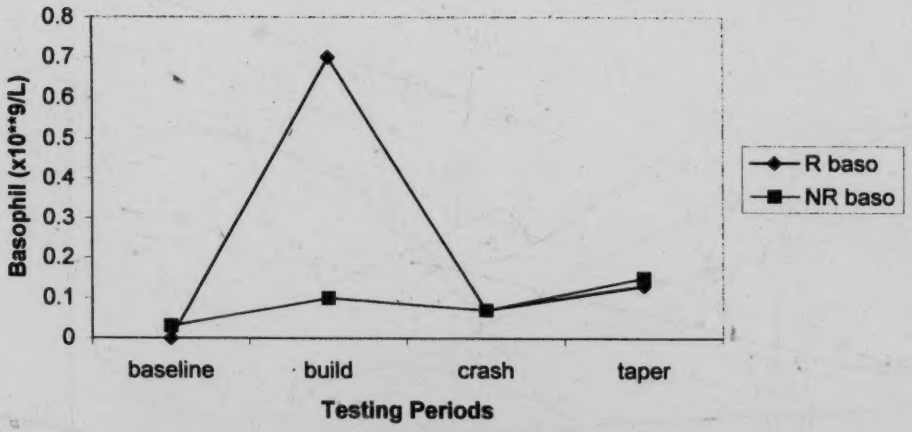

Figure 4.48 Basophil Concentration for Responders and Non-Responders Across Testing Periods

2. T-cells and Natural Killer Cells:

Other immunological variables that were examined for the responders and non-responders were their T-cells and NK cells (Table 4.28). A similar trend for the T-cells for both the responders and non-responders was observed (Figures 4.49 & 4.50). Conversely, Figure 4.51 shows a very different response in terms of NK cells for the responders and non-responders. For the non-responders, the NK cells were at their lowest level during the build phase, but then increased sharply at the crash phase. For the responders, the lowest level occurred during the crash period.

Table 4.28 T-cells (CD4+ and CD8+) and Natural Killer Cells (CD16+CD56+) for Responders versus Non-Responders

Measure	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)	
	R	NR	R	NR	R	NR
Total CD4+ (%)	50	56	59	60	62	64
Total CD8+ (%)	23	28	28	26	27	25
CD4+/CD8+ Ratio	2.3	2.0	2.1	2.3	2.5	2.6
CD16+CD56+ (%)	4.4	3.6	3.2	1.8	0.5	3.7

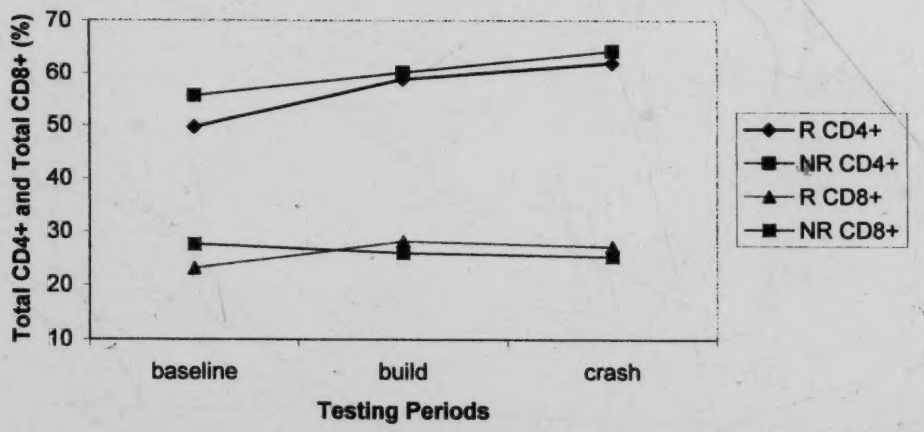

Figure 4.49 Percentage of T-cells for Responders and Non-Responders Across Testing Periods

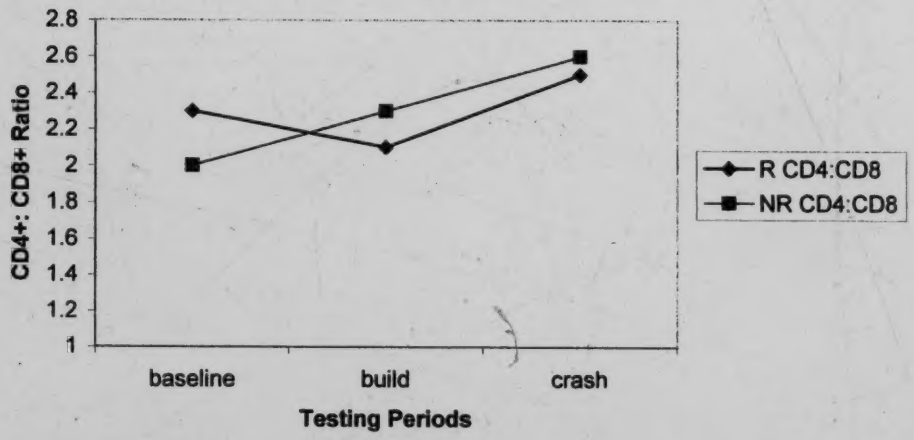

Figure 4.50 CD4+/CD8+ Ratio for Responders Versus Non-Responders Across Testing Periods

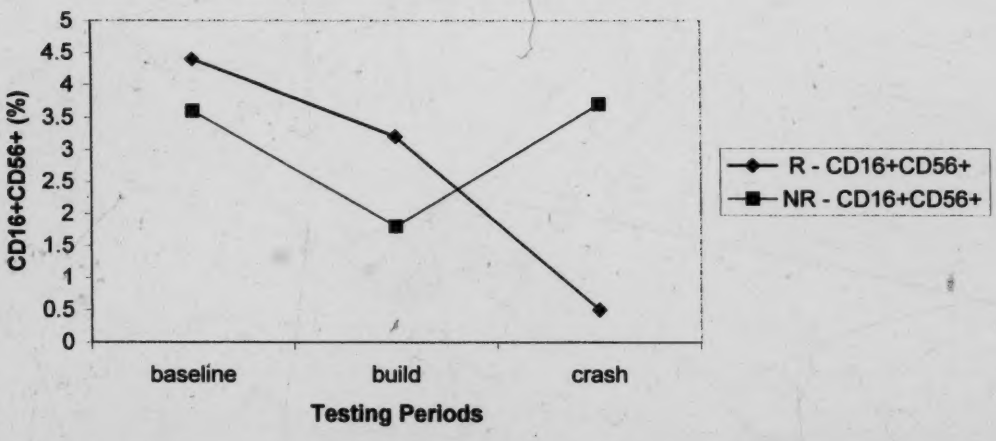

Figure 4.51 Percentage of Natural Killer Cells for Responders and Non-Responders Across Testing Periods

3. Salivary Immunoglobin A:

The results for S-IgA proved to be quite discrepant for the responders and non-responders (Table 4.29, Figure 4.52). For the non-responders, the lowest S-IgA levels were found during the crash period, but for the responders, the crash period had the highest S-IgA levels.

Table 4.29 Salivary Immunoglobin A Levels for Responders and Non-Responders

Measure	**Baseline** (Sept. 9 & 12)		**Build** (Oct. 15th)		**Crash** (Nov. 12th)		**Taper** (Nov. 25th)	
	R	NR	R	NR	R	NR	R	NR
Salivary IgA (ug/ml)	89.3	**76.0**	162.2	**135.0**	232.2	**71.0**	152.4	**106.9**

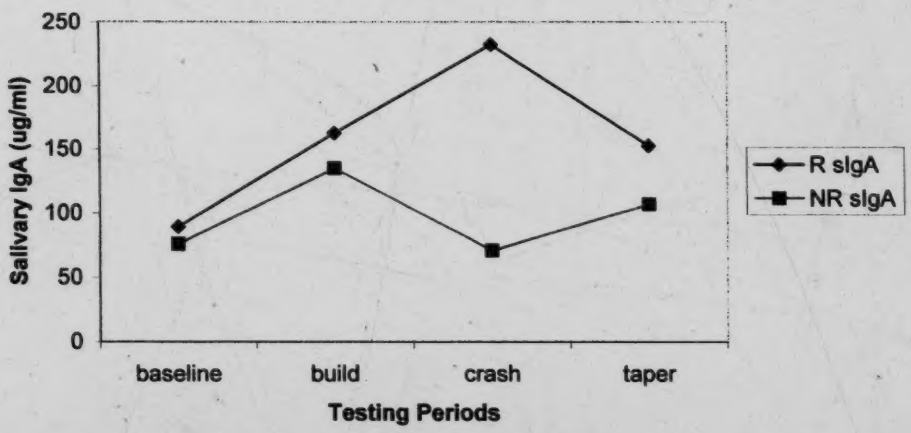

Figure 4.52 Salivary Immunoglobin A Levels for Responders and Non-Responders Across Testing Periods

4. Glutamine and Glutamate:

The final immunological variables that were examined were the amino acids glutamine and glutamate (Table 4.30, Figure 4.53). Although the glutamine:glutamate ratio appears to follow a similar trend for both the responders and non-responders, the ratio is lower for the non-responders at the crash and taper period, due to an increase in glutamate at the crash period, and an increase in glutamate and a decrease in glutamine at the taper period.

Table 4.30 Glutamine and Glutamate Concentrations Across Time for Responders and Non-Responders

Measure	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)	
	R	NR	R	NR	R	NR	R	NR
Glutamate (nmol/L)	66.2	80.4	62.3	55.1	51.9	75.1	62.9	95.7
Glutamine (nmol/L)	963.0	975.2	777.3	798.9	795.6	793.6	640.6	700.9
Glutamine: Glutamate	14.4	12.4	13.2	14.5	15.4	11.7	10.2	7.6

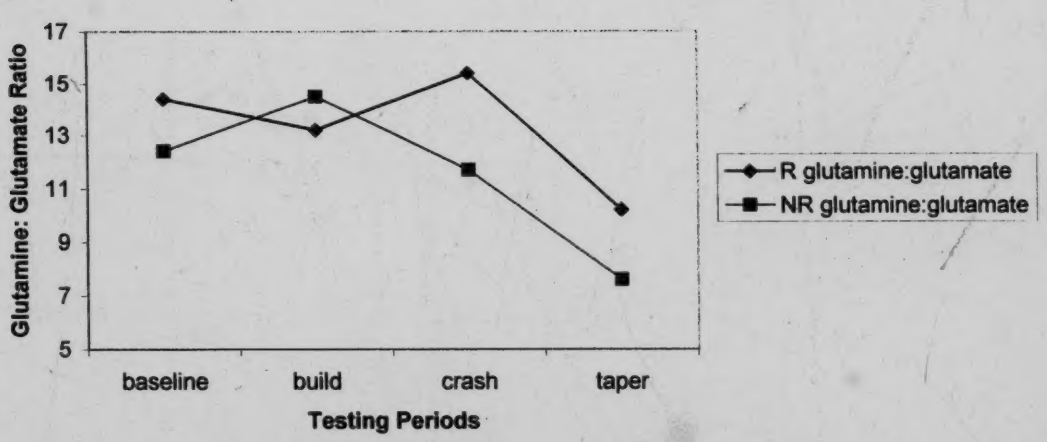

Figure 4.53 Glutamine:Glutamate Ratio for Responders and Non-Responders Across Testing Periods

F) *HORMONAL MEASURE: RESPONDERS VS. NON-RESPONDERS:*

1. Cortisol:

The results for cortisol are difficult to interpret. All 3 of the responders and 1 of the non-responders failed to give their saliva sample for the afternoon or evening cortisol analysis on various occasions throughout the study. Therefore, the results provided in Table 4.31 are the raw data for the 3 responders and the 3 non-responders at each of the test periods for all times of day. Fortunately, all of the responders and all of the non-responders provided their saliva samples during the morning test sessions. Table 4.32 compares the mean results for the morning cortisol for the responders and non-responders. From Figure 4.54, it can be seen that morning cortisol levels were at their lowest level during the taper period for both the responders and non-responders.

Table 4.31 Individual Cortisol Concentrations for the Responders and Non-Responders

Cortisol (nmol/L)		RESPONDERS - subject #			NON-RESPONDERS -subject #		
		02	09	10	01	11	14
Afternoon	Baseline	17.2	9.1	11.4	17.9	12.3	15.6
	Build	1.4	12.8	0	15.2	0.7	33.8
	Crash	5.8	NA	NA	7.5	7.7	11.3
	Taper	NA	NA	6.1	NA	7.8	3.6
Evening	Baseline	8.6	10.8	7.5	5.4	9.7	3.1
	Build	2.8	NA	5.3	NA	9.4	0
	Crash	0	NA	0	1.9	7.2	0
	Taper	0	NA	0	NA	0	0
Morning	Baseline	52	24.3	43.8	34.5	34.5	27.7
	Build	43.5	29.6	49.2	18.5	25.6	35.4
	Crash	48.1	13.0	29.7	29.8	21.1	37.8
	Taper	54.1	0	10.6	11.9	11.0	23.9

Table 4.32 Mean Morning Salivary Cortisol Concentrations for Responders and Non-Responders

Measure (nmol/L)	Baseline		Build		Crash		Taper	
	R	NR	R	NR	R	NR	R	NR
Morning Cortisol (6-8AM)	40.0	32.2	40.8	26.5	30.3	29.6	21.6	15.6

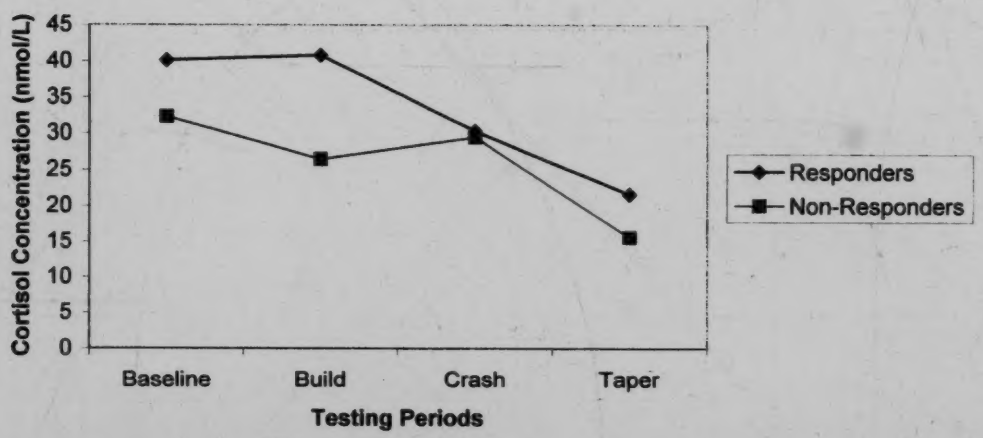

Figure 4.54 Morning (6-8AM) Cortisol Concentrations for Responders and Non-Responders Across Testing Periods

G) CARDIOVASCULAR MEASURES: RESPONDERS VS. NON-RESPONDERS:

Table 4.33 and Figures 4.55 and 4.56 contain the results for the dynamic postural heart rate test (Rusko). Looking at the two graphs together, it is apparent that they follow the same pattern. When comparing the responders to the non-responders, the pattern appears to be fairly similar from the baseline test until the crash test. However, at the taper period, the Rusko score and the average of the last 30 seconds of standing increases more dramatically for the non-responders compared to the responders.

Table 4.33 Results from the Dynamic Postural Heart Rate (Rusko) Test for Responders and Non-Responders

MEASURE	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)	
	R	NR	R	NR	R	NR	R	NR
Average Heart Rate (bpm)	105.2	90.3	91.9	75.9	97.6	81.4	101.5	98.1
Calculated Rusko Score	38.4	32.8	34.0	28.4	35.0	30.2	36.3	34.9

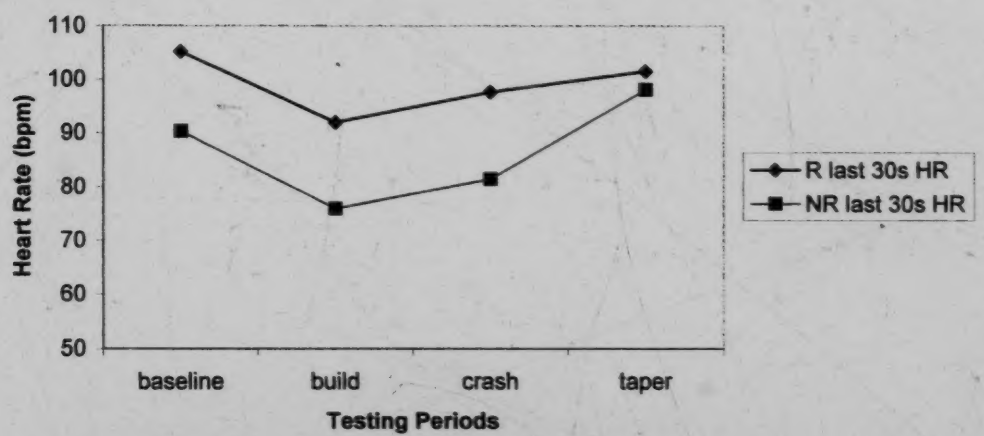

Figure 4.55 Average Heart Rate for the Last 30 seconds of Standing (Rusko Test) for Responders and Non-Responders Across Testing Periods

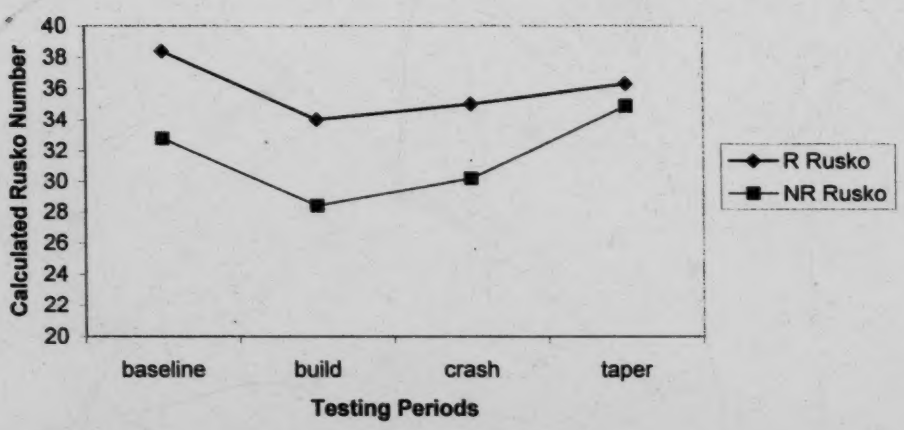

Figure 4.56 Calculated Rusko Number for Responders and Non-Responders Across Testing Periods

In terms of the spectral heart rate analysis, all of the responders and non-responders performed the test at each of the periods. In some situations however, the OmegaWave Sport Technology System fails to provide numerical values for the very low frequency, low frequency and high frequency components of heart rate modulation. This happened to one of the responders during the build test, and also to one of the non-responders during the taper test due to significant heart rhythm disturbances. Therefore, the results in Table 4.34 below only provide the results for 2 of the responders compared to 2 of the non-responders for the spectrum percentages.

Table 4.34 Spectral Heart Rate Analysis Results for Responders and Non-Responders

MEASURE	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)	
	R	NR	R	NR	R	NR	R	NR
Total HRV score n=3	16.8	16.3	16.0	13.3	14.7	16.7	13.0	19.0
VLF Spectrum (%) n=2	10.0	3.9	7.5	14.8	4.0	14.3	5.9	10.1
LF Spectrum (%) n=2	51.3	27.1	45.4	25.0	27.9	24.6	27.0	40.1
HF Spectrum (%) n=2	38.8	69.0	47.1	60.3	68.2	61.1	67.3	49.9
Vagus regulation n=3	0.39	0.33	0.43	0.38	0.45	0.44	0.39	0.43
Sympathetic regulation n=3	24.3	27.7	20.7	24.3	28.7	25.0	20.3	24.7

HRV = heart rate variability, VLF = very low frequency, LF = low frequency, HF= high frequency

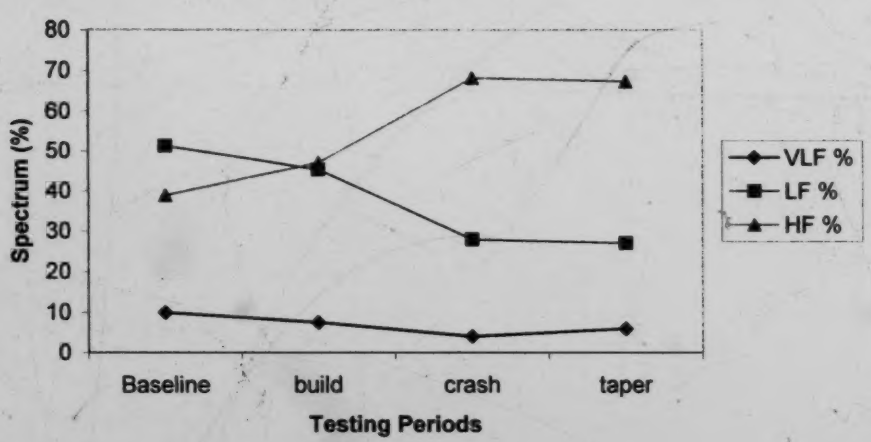

Figure 4.57 Percentage of the Very Low Frequency, Low Frequency and High Frequency Components of Heart Rate for Responders Across Testing Periods

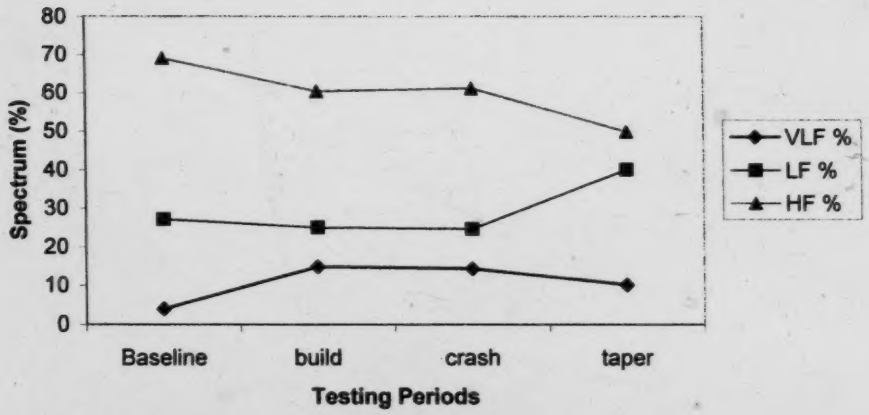

Figure 4.58 Percentage of the Very Low Frequency, Low Frequency and High Frequency Components of Heart Rate for Non-Responders Across Testing Periods

H) *PERFORMANCE MEASURES: RESPONDERS VS. NON-RESPONDERS:*

The final measures examined for the responders and non-responders are the performance test sets and time trials (Table 4.33, Figure 4.54). For the non-responders, it is apparent that these three athletes swam their fastest during the crash period, but then swam much slower during the taper period for both the 100m time trial and the 4 x 50m test set (which gave the predicted 100m time). The responders, on the other hand, did not vary much in terms of race time from the build test to the taper test. It is important to note though, that for these two variables, only the data from 2 of the responders and from 2 of the non-responders is listed below. One of the responders missed performing the tests during the baseline period, and one of the non-responders was unable to complete the tests during the taper period due to illness. Therefore, these subjects' results are not shown because it would have skewed the results if an average was taken, especially since each athlete raced their best stroke for these tests and therefore had very different race times. Of the 2 responders reported below, one athlete swam backstroke, and the other

one swam freestyle. For the non-responders, one of the athletes swam freestyle and the other subject swam butterfly.

Table 4.35 Results for the 100m Time Trial and Predicted 100m Time (based on the 4x50m test set) for Responders and Non-Responders

MEASURE	Baseline (Sept. 12th)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)	
	R n=2	NR n=2	R n=2	NR n=2	R n=2	NR n=2	R n=2	NR n=2
Predicted 100m time	61.9	63.8	60.6	62.9	59.9	61.2	60.3	62.9
100m Time Trial	65.4	67.7	63.7	65.3	63.3	63.9	63.3	66.0

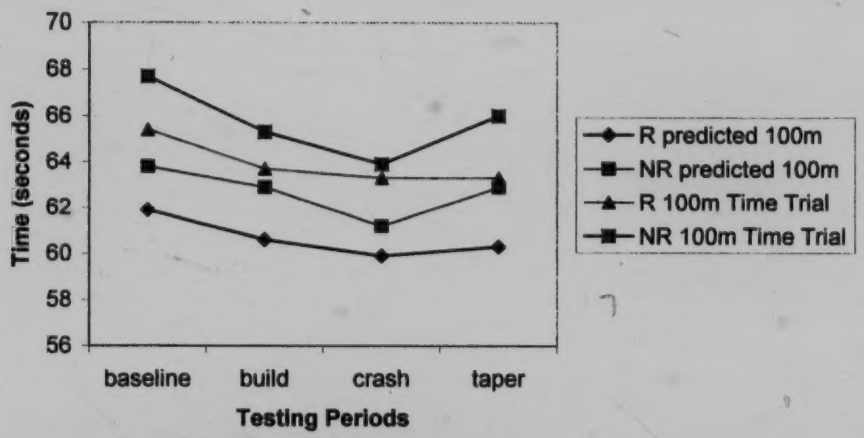

Figure 4.59 Comparison between the 100m Time Trial and the Predicted 100m Time (based on the 4 x 50m Test Set) for Responders and Non-Responders

The responders and non-responders were also asked to complete a 5 x 200m test set at each test period. Unfortunately, one of the non-responders was unable to complete the test during the taper period due to illness, and therefore only the results for the other two non-responders are displayed below. Of the two non-responders listed below, both of the athletes swam freestyle for the test. Of the responders, two swam freestyle, but one athlete swam backstroke for each of the tests. Figure 4.60 illustrates the changes in the predicted 400m time across the study for the responders and non-responders.

Interestingly, the results appear to follow the same pattern for both the responders and non-responders. Figure 4.61 displays the average slope of the heart rate/velocity graph for the responders and non-responders.

Table 4.36 Results from the 5 x 200m Test Set for Responders and Non-Responders

MEASURE	Baseline (Sept. 12th)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)	
	R n=3	NR n=2	R n=3	NR n=2	R n=3	NR n=2	R n=3	NR n=2
Slope of the HR/velocity graph	266.1	473.6	239.5	279.3	233.0	254.0	167.3	257.3
Intercept	-152	-423	-136	-195	-128	-161	-40	-175
r^2	0.95	0.96	0.96	0.98	0.96	0.96	0.96	0.99
Predicted 100m time at HR_{max} (seconds)	74.4	74.9	70.5	69.4	69.8	69.4	68.7	67.6
Predicted 400m time (seconds)	297.7	299.4	282.1	277.4	279.2	277.4	274.7	270.4

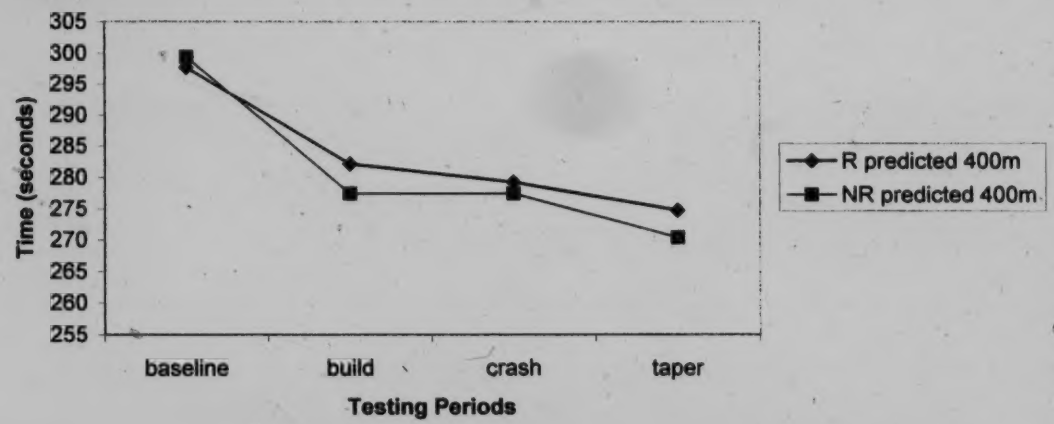

Figure 4.60 Predicted 400m Time for Responders and Non-Responders Across Testing Periods

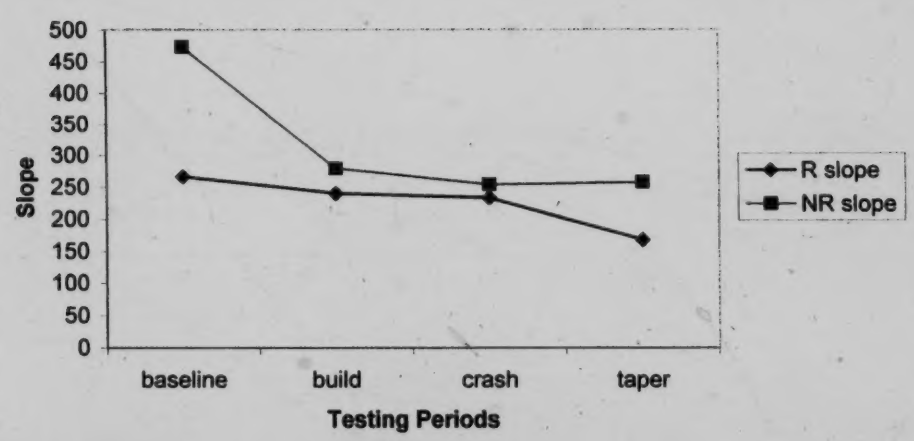

Figure 4.61 Slope of the 5 x 200m Test Set for Responders and Non-Responders Across Testing Periods

CASE STUDY RESULTS: SUBJECT 01

Subject 01 was examined on an individual basis. This subject was chosen based on her profound under-performance (93.7%) at the Canadian National Championships (see Table 4.19). This swimmer swam considerably slower than usual in the two events that she competed in. During her 400m freestyle race, she was 21.3 seconds slower than her best time (a percentage of 92.7), and in her 800m freestyle race she was 31.3 seconds slower than her best time (94.6% of her best). Based on her performance in these two aforementioned races, this athlete scratched from 3 other events.

An attempt has been made to summarize the results for subject 01 over the course of the study. Her data is presented in tables, and graphs have been made to illustrate the physiological changes that occurred over the 10 weeks.

A) *SUBJECT CHARACTERISTICS: SUBJECT 01*

Subject 01 was born on June 19th, 1986, which made this athlete 16 years of age throughout this study. Her average height over the 10-week study was 175.8cm. At the start of the study, her weight was 63.9 Kg, her waist circumference was 70.5cm, and her body mass index (BMI) was 20.7Kg/m^2. Her height, weight, waist circumference and BMI at each testing session can be seen in Table 4.37. It is important to note that this subject recorded her lowest weight, waist circumference and BMI during the taper period.

Table 4.37 Subject Characteristics for Subject 01

Measure	Baseline (Sept. 9 & 12)	Build (Oct. 15th)	Crash (Nov. 12th)	Taper (Nov. 25th)
Height (cm)	175.8	175.3	175.6	175.6
Weight (Kg)	63.9	61.7	62.9	61.3
Waist (cm)	70.5	69	68.5	67.5
BMI (Kg/m^2)	20.7	20.1	20.4	19.9

B) *ENERGY INTAKE: SUBJECT 01*

In terms of her 3-day dietary records, this subject consumed less calories at both testing periods (2559Kcal and 2190Kcal respectively) compared to the average of the other female subjects (3201Kcal and 2559Kcal respectively). Her protein intake as a percentage of total calories (12% and 10%) was lower than the female group mean (13.9% and 13.0%) at both test times. Grams of protein were considerably lower (76g and 56g) than the female group (112g and 84g). Similarly, her fat intake as a percentage of calories (29% and 26%) was lower than the female group mean (29.8% and 30.5%) at both tests. Her fat intake in grams was also significantly lower (86g and 66g) than the female group (112g and 89g). In relation to her carbohydrate intake as a percentage of total calories, she was higher (59% and 64%) than the female group (56% and 56%) at both test times. However, she ingested

fewer grams of carbohydrate (384g) than the female group (444g) at the first test period, and was relatively similar to the group at the second test period (363g compared to the group mean of 361g).

C) *TRAINING: SUBJECT 01*

This swimmer was a chronically trained athlete who could be classified as a distance swimmer. She typically trained 6 days/week for 11 months of the year. This usually equated to 18+ training hours per week. Over the course of the 10 week study her workouts were of varying length. Generally, subject 01 had similar swim volumes compared to the group mean for weeks 1-6, however beginning in week 7, she had a consistently higher volume of training (especially compared to week 6). Week 7 was a considerably high-volume training week, with 70 Km swum, and 195 minutes of dryland training (Table 4.38).

Table 4.38 Comparison of Weekly Training between the group mean and Subject 01

TRAINING DATES	Swim Volume (Km)		Dryland time (min.)	
	Group mean n=14	Subject 01	Group mean n=14	Subject 01
Week 1: Sept. 16 - 22	12.0	11.3	128.9	240.0
Week 2: Sept. 23 - 29	16.9	17.3	120.6	165.0
Week 3: Sept. 30 - Oct. 6	30.6	29.3	112.8	120.0
Week 4: Oct. 7 - 13	39.1	44.4	128.9	180.0
Week 5: Oct. 14 - 20	37.9	40.3	65	90.0
Week 6: Oct. 21 - 27	32.8	29.1	51.3	110.0
Week 7: Oct. 28 - Nov. 3	44.7	70.0	70	195.0
Week 8: Nov. 4 - 10	44.9	46.6	81.9	105.0
Week 9: Nov. 11 - 17	36.4	53.0	58.3	180.0
Week 10: Nov. 18 - 24	30.0	52.0	45.7	165.0

Reminder: baseline test occurred before the start of training, the build test took place after week 4 (Oct.15th), the crash test occurred after week 8 (Nov.12th), and the taper test happened after week 10 (Nov.25th).

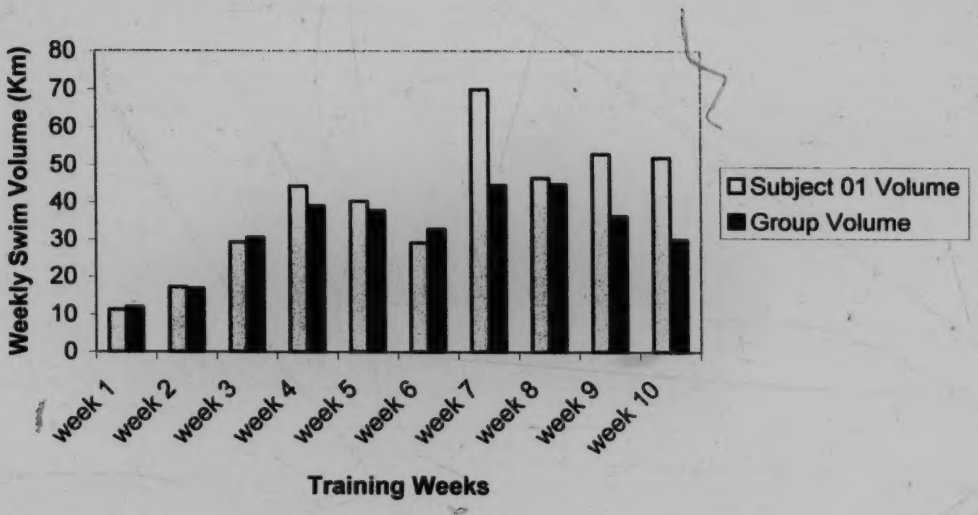

Figure 4.62 Weekly Swim Volume for Subject 01 and for the Group

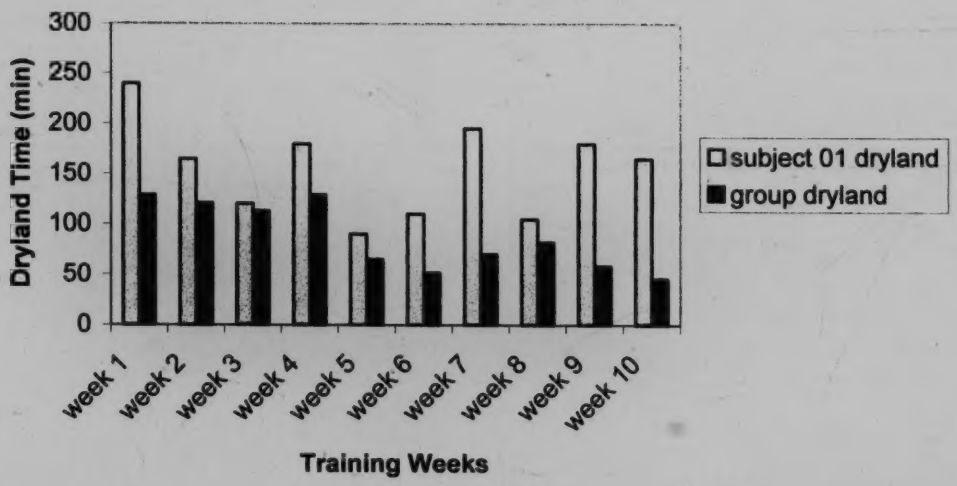

Figure 4.63 Time Spent doing Dryland Activities/Week for Subject 01 and for the Group

A summary of Subject 01's subjective ratings of fatigue and intensity for each of the 10 weeks can be found in Table 4.39. The group means are also included for comparison.

Table 4.39 Comparison of Subjective Ratings of Training Intensity and Fatigue between the group mean and Subject 01

TRAINING DATES	Training Intensity		Fatigue	
	Group mean n=14	Subject 01	Group mean n=14	Subject 01
Week 1: Sept. 16 - 22	2.4	2.25	2.6	2.75
Week 2: Sept. 23 - 29	3.8	3.5	3.6	3.5
Week 3: Sept. 30 - Oct. 6	3.6	3.2	3.7	3.3
Week 4: Oct. 7 - 13	3.9	3.1	4.0	3.6
Week 5: Oct. 14 - 20	4.5	4.2	4.6	5.0
Week 6: Oct. 21 - 27	4.1	3.0	4.3	2.4
Week 7: Oct. 28 - Nov. 3	4.5	4.3	5.3	4.7
Week 8: Nov. 4 - 10	4.4	4.1	4.7	4.7
Week 9: Nov. 11 - 17	4.5	4.3	4.4	4.3
Week 10: Nov. 18 - 24	4.0	4.1	4.2	4.9

Reminder: baseline test occurred before the start of training, the build test took place after week 4 (Oct.15th), the crash test occurred after week 8 (Nov.12th), and the taper test happened after week 10 (Nov.25th).

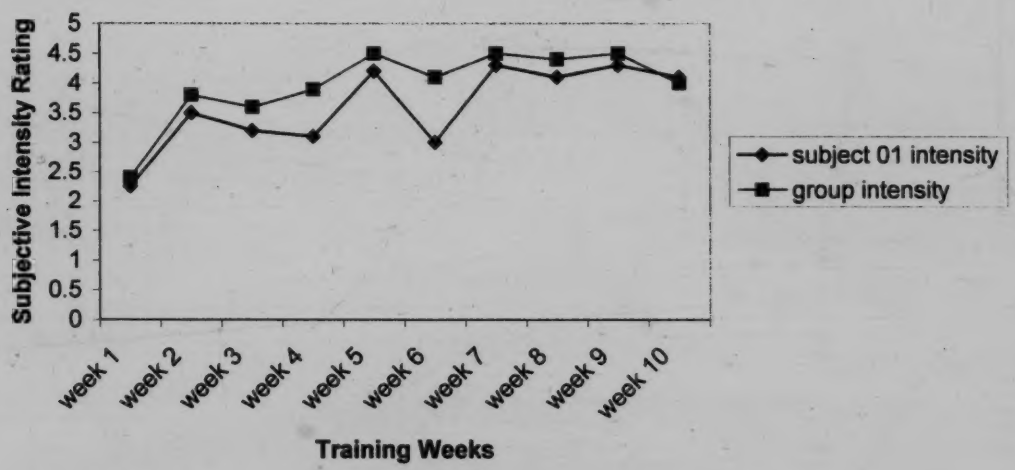

Figure 4.64 Weekly Subjective Ratings of Training Intensity for Subject 01 and for the Group

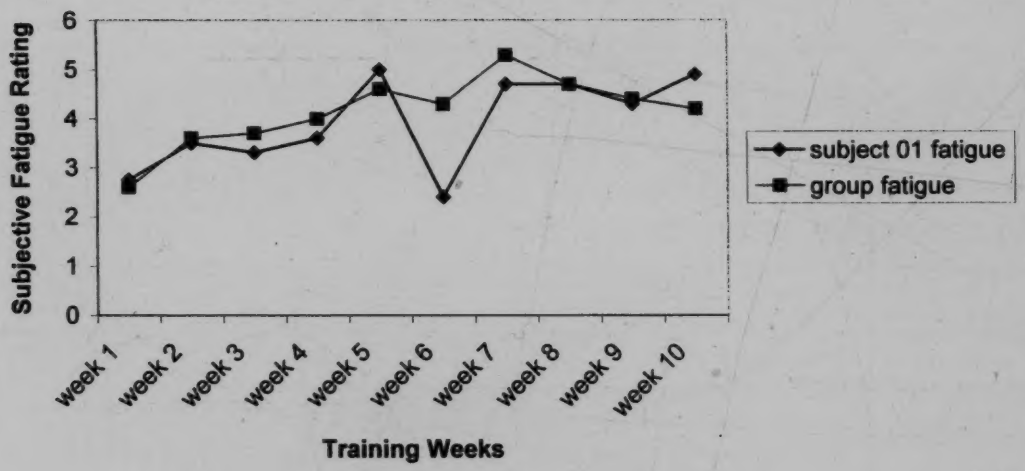

Figure 4.65 Weekly Subjective Ratings of Fatigue for Subject 01 and for the Group

Based on her training log book, information was also obtained for her frequency of illnesses, and the occurrence of menstruation. In terms of illnesses, subject 01 reported that she was sick at 19 out of a total of 77 practices (24.7%) that she recorded. This is higher than the group mean of 11.9%. In relation to menstruation, Table 4.6 shows that her period lasted 5 days on average, and she had her period twice over the course of the study. This appears to be fairly similar to the other female subjects, however subject 01 mentioned in a personal communication that her occurrence of menstruation was fairly irregular.

D) *HEMATOLOGICAL MEASURES: SUBJECT 01*

Results for subject 01 for all of the tests that related to red blood cells can be found in Table 4.40. These results appear fairly similar to the group results that can be observed in Table 4.7. As well, from a visual perspective, these results did not change

significantly across the test periods, and therefore graphs have not been created for these variables.

Table 4.40 Tests Relating to Red Blood Cells for Subject 01

Measure	Baseline (Sept. 9 & 12)	Build (Oct. 15th)	Crash (Nov. 12th)	Taper (Nov. 25th)	Female reference range *
Hemoglobin (g/L)	141	134	136	142	120-160
Red blood cell count ($*10^{12}$/L)	4.75	4.42	4.46	4.66	4.1-5.1
Hematocrit (L/L)	0.42	0.4	0.4	0.42	0.36-0.46
Mean Cell Volume (fL)	89	90	89	89.0	78-100
MCHC (g/L)	337	337	340	340	310-360

* normal reference range provided by the Dynacare Kasper Laboratory, Edmonton (2002)

In terms of iron and its associated variables including ferritin and TIBC a comparison is made between subject 01 and the group means, as subject 01 appears to be significantly low for these variables at each time point (Table 4.41, Figures 4.66, 4.67 and 4.68). More specifically, subject 01's iron level at the crash and taper periods was below the female reference range (8-25umol/L) as outlined by the Dynacare Kasper Medical Laboratory.

Table 4.41 Comparison of tests of iron status for subject 01 and group mean

Measure	Female reference range *	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)	
		group n=14	# 01	group n=14	# 01	group n=14	# 01	group n=14	# 01
Iron (umol/L)	8-25	20.4	11	13.9	13	15.3	4	16.4	4
TIBC (umol/L)	40-80	57.9	55	53.9	51	57.2	52	58.1	49
Ferritin (ug/L)	12-300	79.8	35	45.9	18	51.2	26	56.9	33

Note: values are bolded if they are outside of the normal reference range
* normal reference range provided by the Dynacare Kasper Laboratory, Edmonton (2002)

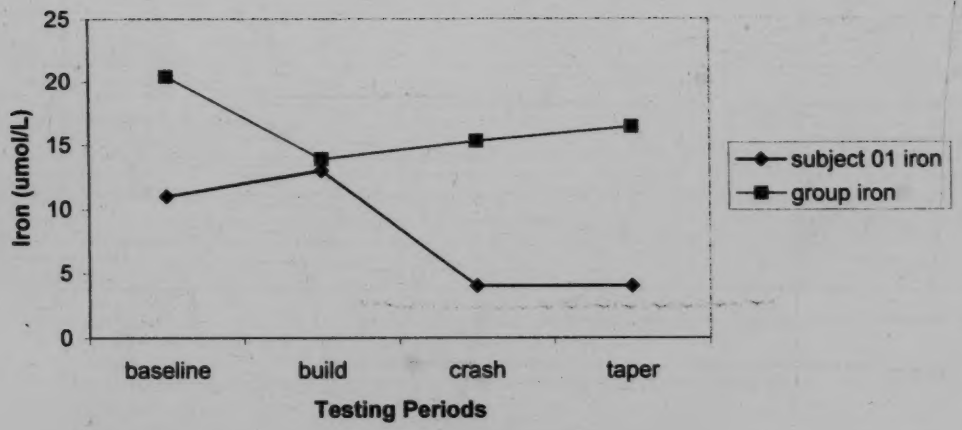

Figure 4.66 Comparison of Iron Levels for Subject 01 and the Group

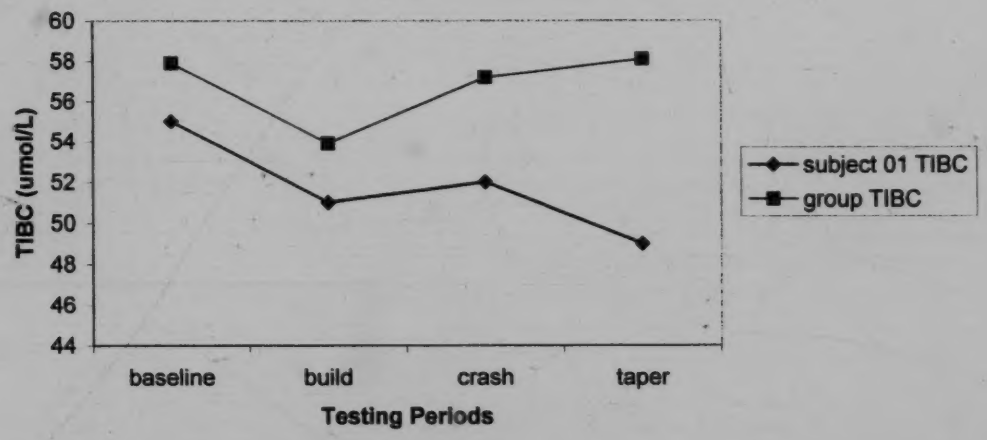

Figure 4.67 Comparison of Total Iron Binding Capacity (TIBC) for Subject 01 and the Group

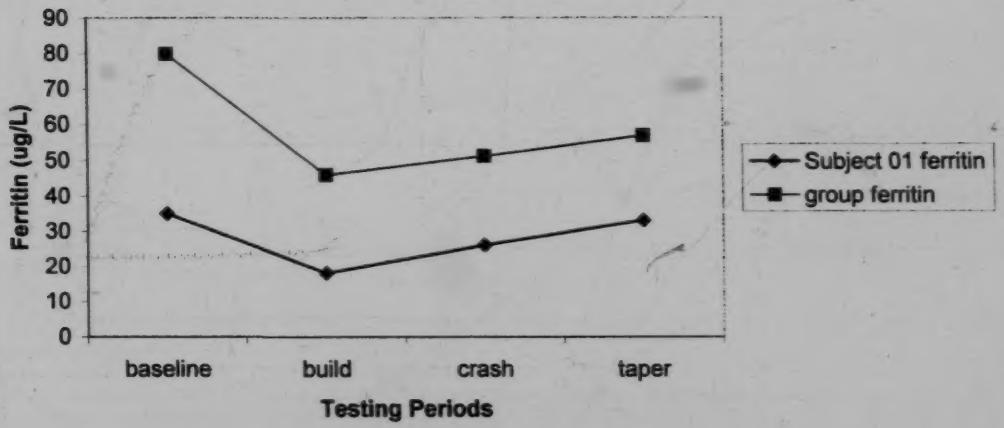

Figure 4.68 Comparison of Ferritin Levels for Subject 01 and the Group

E) *IMMUNOLOGICAL MEASURES: SUBJECT 01*

Results for the white blood cells and differential for subject 01 can be found in Table 4.42. Unfortunately, for this subject the results for eosonophils and basophils are missing for the taper test. Subject 01's neutrophil concentration was below the normal reference range during the build period. Further, subject 01's WBC count and lymphocyte concentration was below the normal reference range during the taper test.

1. White Blood Cells and Differential:

Table 4.42 Results for white blood cells and differential for Subject 01

Measure	**Baseline** (Sept. 9 & 12)	**Build** (Oct. 15th)	**Crash** (Nov. 12th)	**Taper** (Nov. 25th)	**Normal reference range** *
White blood cell ($x10^9$/L)	6.3	4.7	9.1	4.2	4.5-13.0
Neutrophil ($x10^9$/L)	3.1	1.6	5.3	2.5	1.8-8.0
Lymphocyte ($x10^9$/L)	2.4	2.6	1.5	0.8	1.2-5.2
Monocyte ($x10^9$/L)	0.5	0.3	1.8	0.3	0.0-1.0
Eosonophil ($x10^9$/L)	0.1	0.1	0.4	NA	0.0-0.7
Basophil ($x10^9$/L)	0.1	0.1	0.1	NA	0.0-0.3

Note: values are bolded if they are outside of the normal reference range

* normal reference range provided by the Dynacare Kasper laboratory, Edmonton (2002)

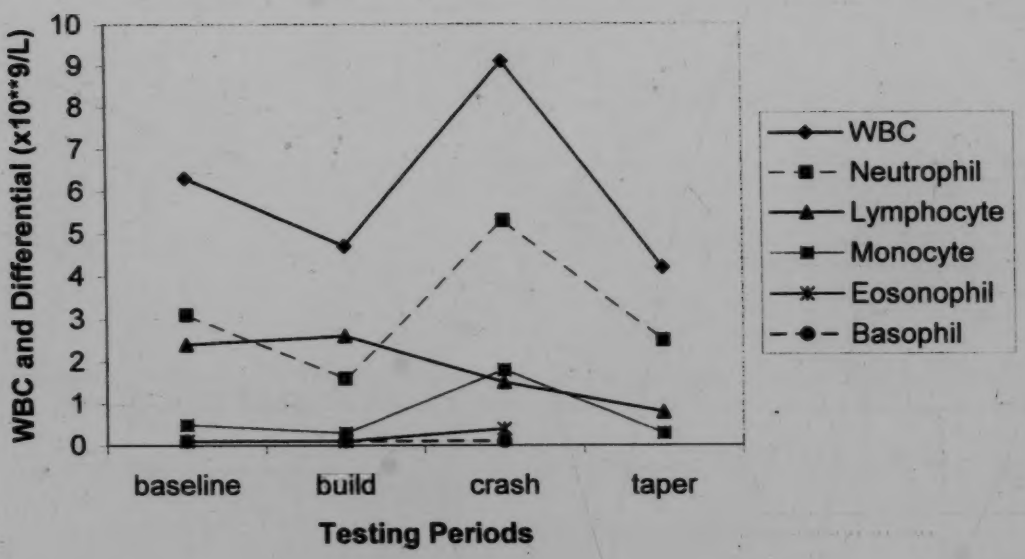

Figure 4.69 Subject 01's White Blood Cell and Differential Results

2. T-cells and Natural Killer Cells:

Results for subject 01's T-cells and natural killer cells can be found in Table 4.43. As was previously mentioned, there are only results for the baseline, build and crash periods. The CD4+/CD8+ ratio decreased during the crash period for subject 01, but increased during the crash for the group as a whole (Figure 4.70). Subject 01's natural killer cells increased significantly during the crash period compared to the group (Figure 4.71).

Table 4.43 T-cells (CD4+ and CD8+) and Natural Killer Cells (CD16+CD56+) for Subject 01

Measure	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)	
	group n=14	subject 01	group n=14	subject 01	group n=14	subject 01
Total CD4+ (%)	49.6	52.55	56.2	60.0	58.5	55.09
Total CD8+ (%)	27.7	31.01	27.9	27.63	26.6	29.9
CD4+/CD8+ Ratio	1.9	1.69	2.0	2.17	2.3	1.84
CD16+CD56+ (%)	5.7	3.47	5.1	1.8	3.7	8.1

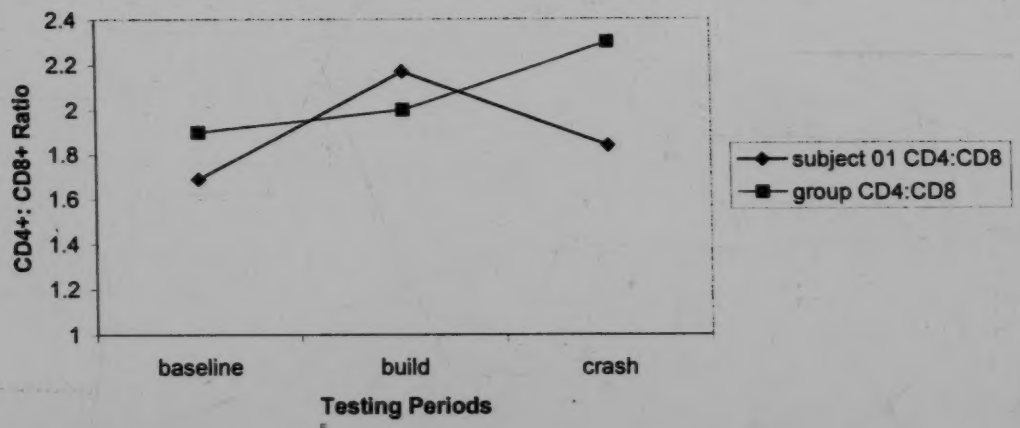

Figure 4.70 Subject 01's CD4+/ CD8+ Ratio Across Testing Periods Compared to the Group

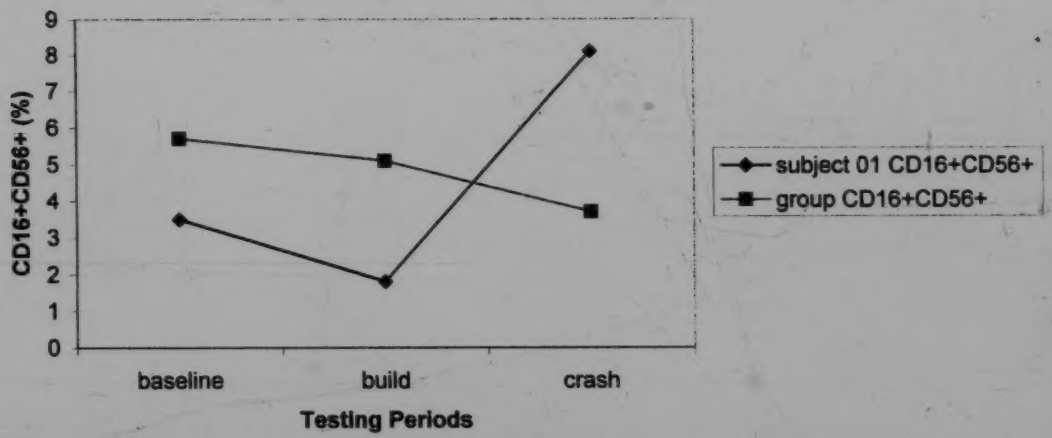

Figure 4.71 Comparison of Natural Killer Cells between Subject 01 and the Group

3. Salivary Immunoglobin A:

The results for S-IgA demonstrated that subject 01 had significantly lower levels than the group mean at all testing times (Table 4.44, Figure 4.72).

Table 4.44 Comparison of Salivary Immunoglobin A for Subject 01 with Group Mean

Measure	Baseline (Sept. 9 & 12)		Build (Oct. 15th)		Crash (Nov. 12th)		Taper (Nov. 25th)	
	group n=14	subject 01	group n=14	subject 01	group n=14	subject 01	group n=14	subject 01
Salivary IgA (ug/ml)	93.8	43.25	158.8	66.5	128.1	58.63	118.0	60.71

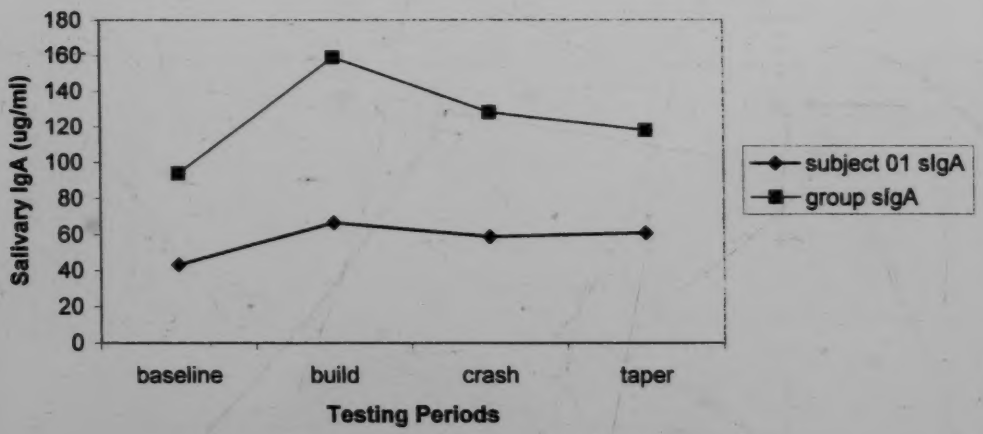

Figure 4.72 Salivary IgA levels for Subject 01 and the Group

4. Glutamine and Glutamate:

The final immunological measures to be examined for subject 01 were glutamine and glutamate (Table 4.45, Figure 4.73). It is clear that the ratio decreased from the build period through to the taper.

Table 4.45 Glutamine and Glutamate Concentrations Across Time for Subject 01

Measure	Baseline (Sept. 9 & 12)	Build (Oct. 15th)	Crash (Nov. 12th)	Taper (Nov. 25th)
Glutamate (nmol/L)	77.23	63.29	82.49	110.71
Glutamine (nmol/L)	945.15	891.74	719.46	565.13
Glutamine: Glutamate	12.24	14.09	8.72	5.10

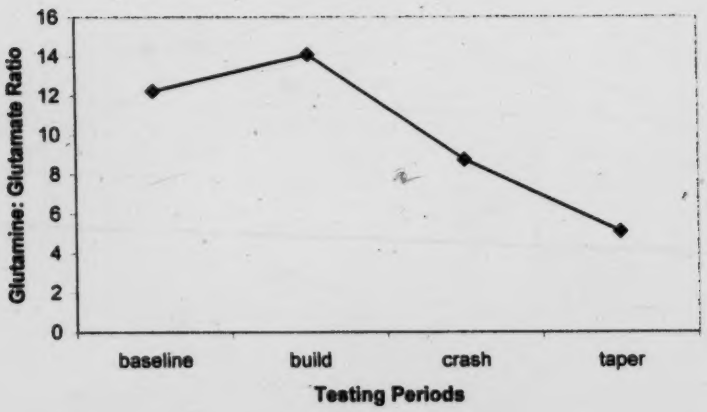

Figure 4.73 Glutamine: Glutamate Ratio for Subject 01 Across Testing Periods

F) *HORMONAL MEASURE: SUBJECT 01*

Salivary cortisol levels were also examined separately for subject 01. Unfortunately values are missing for the evening during the build period, as well as for the evening and afternoon cortisol during the taper period. There appears to be no clear pattern regarding subject 01's cortisol concentrations (Table 4.46 and Figure 4.74).

Table 4.46 Salivary Cortisol Concentrations for Subject 01

Measure (nmol/L)	Baseline	Build	Crash	Taper
Afternoon Cortisol (4-5PM)	17.9	15.2	7.5	NA
Evening Cortisol (8-10PM)	5.4	NA	1.9	NA
Morning Cortisol (6-8AM)	34.5	18.5	29.8	11.9

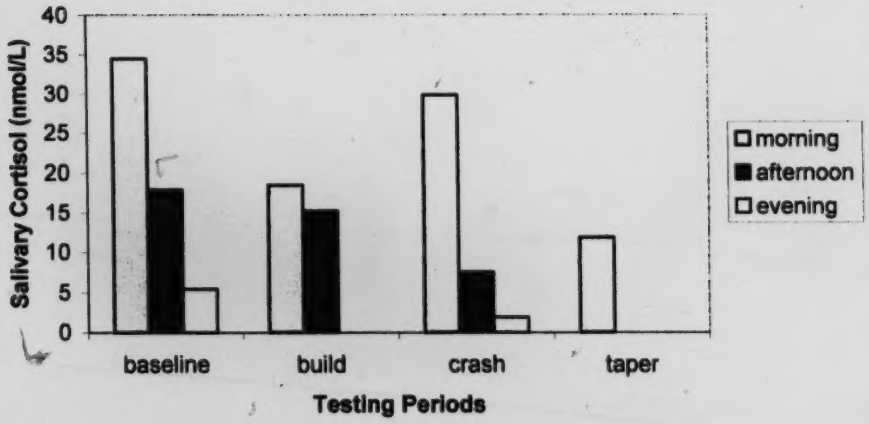

Figure 4.74 Subject 01's Cortisol Concentrations Across Testing Periods

G) *CARDIOVASULAR MEASURES: SUBJECT 01*

The results for subject 01's cardiovascular measures proved to be interesting as well, as changes appeared to occur over the 10-week study. In terms of the dynamic postural heart rate test (Rusko), the entire heart rate record for the 10 minute test can be seen in Figure 4.75 (reminder: standing took place at 480 seconds). From the graph, it can be seen that the highest heart rate response to standing occurred during the taper period. This information corresponds with the fact that the average heart rate over the last 30 seconds of standing was highest during the taper, and also with the fact that the calculated Rusko score was the highest at that time point as well (Table 4.47).

Table 4.47 Dynamic Postural Heart Rate (Rusko) Test Results for Subject 01

MEASURE	Baseline (Sept. 9 & 12)	Build (Oct. 15th)	Crash (Nov. 12th)	Taper (Nov. 25th)
Average Heart Rate (bpm)	78.2	53.2	83.3	106.0
Calculated Rusko Score	28.3	20.3	30.5	35.9

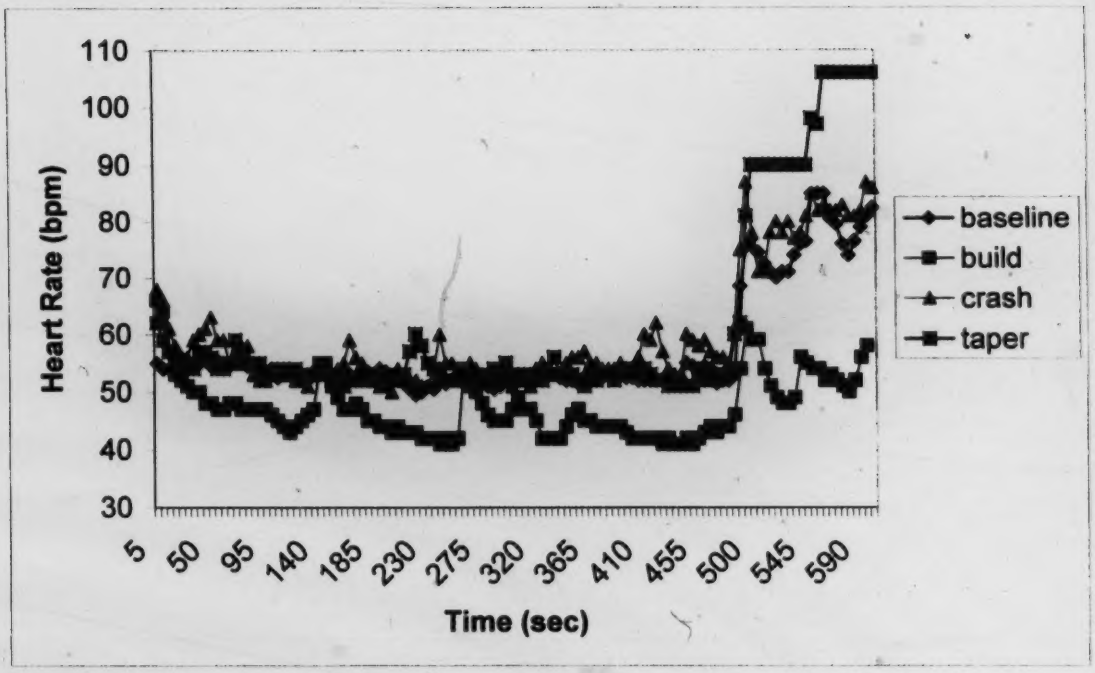

Figure 4.75 Heart Rate Record for the Dynamic Postural Heart Rate Test (Rusko) for Subject 01

Subject 01 also performed the spectral heart rate analysis at each of the test periods. Most notable is the fact that the percentage of high frequency modulation increased during the taper period. An increase in the high frequency component represents an increase in vagal modulation (see Table 4.48 and Figure 4.76).

Table 4.48 Results from the Spectral Heart Rate Analysis for Subject 01

MEASURE	Baseline (Sept. 9 & 12)	Build (Oct. 15th)	Crash (Nov. 12th)	Taper (Nov. 25th)
Total HRV score	12	11	19	17
VLF Spectrum (%)	5.1	27.8	25.3	10.3
LF Spectrum (%)	35.95	27.0	29.7	28.0
HF Spectrum (%)	58.95	45.2	44.9	61.7
Vagus Regulation	0.21	0.31	0.51	0.31
Sympathetic Regulation	34.0	26.0	24.0	35.0

HRV = heart rate variability, VLF = very low frequency, LF = low frequency, HF= high frequency

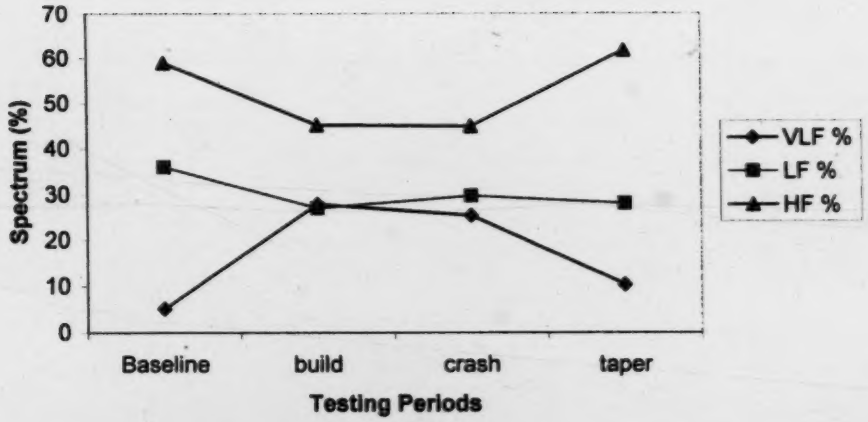

Figure 4.76 Percentage of the Very Low Frequency, Low Frequency and High Frequency Components of Heart Rate for Subject 01

H) ***PERFORMANCE MEASURES: SUBJECT 01***

As a final examination of subject 01, her performance in the swimming test sets was examined. For both the 100m time trial and the predicted 100m (based on the 4 x 50m test set), subject 01's results were almost the same at the baseline time period (before she had started training) as they were during the taper time period (Table 4.49, Figure 4.77).

Table 4.49 Results from the 100m time trial and Predicted 100m time (based on the 4 x 50m test set) for Subject 01

MEASURE	**Baseline** (Sept. 12th)	**Build** (Oct. 15th)	**Crash** (Nov. 12th)	**Taper** (Nov. 25th)
Predicted 100m time	1:06.3	1:05.9	1:03.2	1:06.6
100m Time Trial	1:11.2	1:08.7	1:06.0	1:10.0

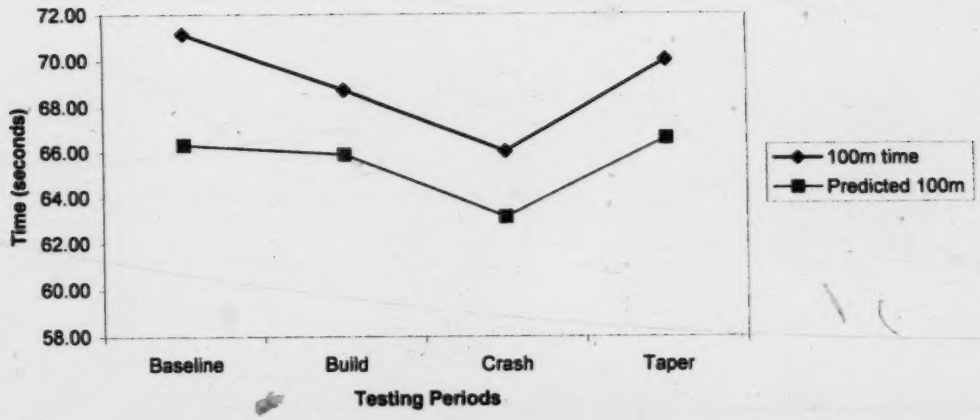

Figure 4.77 Comparison between the 100m Time Trial and the Predicted 100m Time for Subject 01

Further, Table 4.50 displays her results in the 5 x 200m test set, both in terms of a predicted 400m time, and the slope of the 5 x 200m heart rate/velocity graph. For this test, subject 01 swam freestyle. It can be seen that at the start of the season (baseline) subject 01 had a much slower predicted 400m time that at any of the other three tests.

Table 4.50 Results from the 5 x 200m test set for Subject 01

MEASURE	Baseline (Sept. 12th)	Build (Oct. 15th)	Crash (Nov. 12th)	Taper (Nov. 25th)
Slope of the HR/velocity graph	262.5	252.6	255.7	262.2
Intercept	-152.6	-165.3	-166.2	-179.9
r^2	0.98	0.99	0.92	0.99
Predicted 100m time at HR_{max} (seconds)	74.4	69.1	69.8	69.0
Predicted 400m time (seconds)	297.6	276.4	279.2	276.0

As well, to provide a more complete graphical illustration, the actual heart rate/velocity graph has been included for each of the tests (Figure 4.78). As can be seen from the graph, the slope of each of the tests stayed fairly consistent, but shifted to the right.

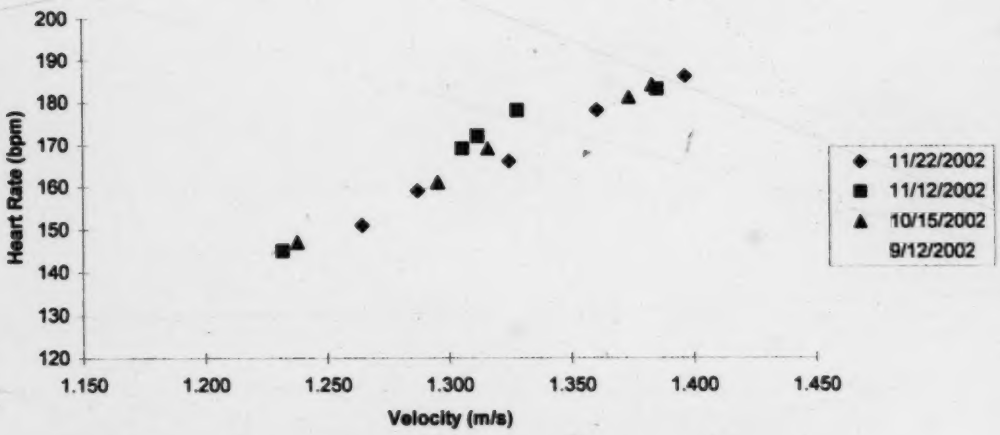

Figure 4.78 Subject 01's Heart Rate/Velocity Graph for the 5 x 200m Test Set

I) *CONCLUSION*

To summarize, this chapter has analyzed the group means for all of the variables examined in this study. Further, repeated measures ANOVAs were run on the group data to determine whether differences existed across the 4 test periods (baseline, build, crash and taper). Next, the subjects were separated into 3 groups, and the extremes were then classified as responders and non-responders to training. Descriptive statistics and graphs were used to compare the groups. Finally, a case study approach was used to examine one outlying subject for all of the study variables based on her under-performance at the major competition.

CHAPTER FIVE: DISCUSSION

OVERVIEW:

This chapter discusses the results for the measures that were outlined in chapter 4 and attempts to provide explanations for the physiological changes that occurred in the 14 subjects. This discussion provides insight into the changes that took place across a training season. Pre-training (baseline) values are examined and compared to training responses (during the build period as well as during the crash period). Further, this chapter looks at the effects of a taper on the study variables.

After the group results are discussed, an attempt is made to delineate the normal changes that occurred with training from the abnormal changes that may be associated with overtraining. In order to accomplish this, the results for the subjects who achieved 100% best times at their major competition (RESPONDERS, n=3) are discussed and compared to the subjects who achieved an average of less than 97% of best times (NON-RESPONDERS, n = 3). It is assumed for the purpose of this study that the non-responders were over-reached and/or overtrained based on their extreme under-performance at their major competition. This assumption is based on the fact that a decrement in performance is the best indicator of overtraining (Uusitalo, 2001).

Finally, this discussion focuses on the results of one case study subject. It was deemed pertinent to look at her information separately in order to see whether any relationship could be drawn between her under-performance and the hematological, immunological, hormonal, cardiovascular and performance measures that were utilized in this study. Further, two types of overtraining states have been identified: sympathetic and parasympathetic (Uusitalo, 2001). It has been speculated that the sympathetic type

represents an early transitory stage that shifts to the parasympathetic type after an individually varied amount of time (Urhausen et al, 1995). In terms of this study, it is believed that the case-study athlete may have been at a different stage of overtraining compared to the other non-responders, and therefore her results are examined separately.

DISCUSSION OF GROUP RESULTS

A) *SUBJECT CHARACTERISTICS:*

Competitive swimmers were an ideal group to study, as research has shown that the prevalence of overtraining is highest in endurance sports requiring high volume intense training, such as swimming (Mackinnon, 2000). The mean age of the subjects in this study was 18.3 ± 0.6 years, which is similar to several other studies that have examined overtraining in competitive swimmers (Hooper et al, 1993; Mujika et al, 1996; Kirwan et al, 1988; Gleeson et al, 1999). Furthermore, all of the subjects who participated in this study had reached puberty. The similarity in age and maturation level of the subjects made for appropriate comparisons with current literature (Tremblay & Chu, 1994).

In terms of height, the results indicated that there was a significant difference across testing periods for both the males and females. However, from looking at the actual results in Table 4.1, it is apparent that the differences were very minor, and therefore it can be concluded that the subjects were not growing in height. The fact that *post hoc* analysis revealed that there were no differences between specific time points supports this conclusion.

Weight, waist circumference and BMI were also statistically significant across time for the male subjects (but not for the females). Specifically, the male subjects decreased

in weight, waist circumference and BMI with each testing period. This would imply that the male subjects were not meeting their energy demands for training (which is not necessarily a negative physiological effect if the male swimmers had put on excess weight over the off season). This finding is reflected in the diet record information that is discussed in the next section.

B) ***ENERGY INTAKE:***

Achieving energy balance is essential for competitive athletes for the maintenance of lean tissue mass, immune and reproductive function, and optimal athletic performance (American Dietetic Association and the Dieticians of Canada, 2000). According to the American Dietetic Association and the Dieticians of Canada, the usual energy intakes for male endurance athletes range from 3000 to 5000 Kcal per day. For female endurance athletes, the usual energy intakes may match those of male athletes per Kg of body weight. Based on the above recommendations from the American Dietetic Association and the Dieticians of Canada, the male athletes were not consuming enough calories/day as they were unable to maintain their weight (4283 Kcal/day during the build period compared to 3717 Kcal/day during the crash period). Although the female athletes did not lose weight over the course of the study, it is interesting that their caloric intake was lower during the period of very hard training compared to the build period (2559 Kcal/day vs. 3201 Kcal/day). Perhaps the athletes need to be educated regarding the importance of energy balance (e.g. energy in = energy out), especially during periods of very hard training. According to Sherman & Maglischo (1991), swimmers who do not voluntarily increase energy consumption to equal the metabolic demands of increased swim training volume/intensity do

not maintain muscle glycogen levels, have reduced training capabilities, and may suffer from chronic athletic fatigue.

The results of this study demonstrate that the female athletes were consuming significantly lower amounts of carbohydrate (361 vs. 444g) and protein (84 vs. 112g) during the crash dietary recording as opposed to the build period recording, respectively. The decrease in protein intake during the crash period is a matter of concern, as inadequate intake of protein impairs host immunity, with particularly detrimental effects on the T-cell system, increasing the risk of infections (Gleeson et al, 2000). The decrease in carbohydrates is also a concern because swimming requires the use of muscle glycogen, and therefore, lowered carbohydrate reserves might contribute to a deterioration in performance during athletic training or competition (Sherman & Maglischo, 1991). As well, carbohydrate is an important fuel for cells of the immune system including lymphocytes, neutrophils and macrophages (Gleeson & Bishop, 2000). Therefore, adequate carbohydrate intake is required to maintain immunocompetence.

C) *TRAINING:*

1. Description of Training

Based on the results for swim volume, amount of dryland activity, and subjective ratings of training intensity and fatigue, it appears as though some planning /coaching errors may have occurred. Training programs need to be structured in such a way as to allow an adequate balance of training stress and recovery (Kreider et al, 1998). This concept is the basis for periodized training. According to Fry et al (1992), a training year should be broken down into 4 major periods termed macrocycles, which are in turn divided

into a number of mezocycles, which are normally 4 weeks in duration. Within each mezocycle there are usually two weeks of development work consisting of increasing volume and intensity (this is also known as a build period) that are followed by one week of intensified volume and intensity (termed the crash phase), and ending with one week of regeneration. Although this is merely a recommended training program, the results from this study do not appear to follow this suggestion. From examining the volume of swim training, the amount of time spent doing dryland activities, and the subjective intensities of the training weeks, it appears as though there was not enough regeneration time included into the training program. It is essential that adequate regeneration time be included in the training programs so that adaptation can be achieved (Fry et al).

Further, the results of this study are concerning due to the fact that the overload period was performed only two weeks prior to the major competition. Norris (2003, personal communication) recommends that a period of very hard training be performed at least 4 weeks prior to a major competition and that peak volume within the macrocycle should be 6-7 weeks from the major competition. Fry et al (1992) suggests that the length of the rest period, following overload training, depends on the time needed to complete the rehabilitative processes, which in turn depends on the degree of fatigue induced by the training program. As well, if the resting phase between two stimuli is either too short or too long, a training effect will not be achieved (Fry et al).

Lastly, there is a concern regarding the taper process that took place during weeks 9 and 10 of this study. An effective taper allows supercompensation processes to maximize the positive effects of training and recovery processes to eliminate fatigue and other negative effects of training (Hooper et al, 1999). As was previously mentioned, the length

of the taper was longer (i.e. averaging approximately 2 weeks vs. 1 week) for the sprinters and middle-distance as compared to the distance swimmers. This resulted in the greatest decrease in volume for the sprinters. Unfortunately, the swimmers were not separated as sprinters, middle-distance or distance swimmers when the weekly swim volume averages were calculated. This is because some of the swimmers trained in more than one category, and it would not be possible to separate the categories with any degree of accuracy. As a whole, the subjects' weekly swim volume was 30.0 Km/week for the week immediately prior to the competition (week 9). This represents a 33.2% reduction in volume from week 8 (which was the 2nd week of the overload training period). Houmard & Anderson Johns (1994) suggest that a taper which improves performance involves a substantial (60-90%) gradual reduction in swim training volume, and daily high intensity interval work over a 7 to 21 day period. A reduction in volume of 33.2% is much less than the recommended 60-90%. Therefore, the subjects in this study were likely performing too much volume prior to the major competition, and this may explain some of the poor competition results that will be discussed later in this chapter.

2. Frequency of Illness:

The mean group results suggest that the subjects were ill 11.9% of the workouts that they attended (Table 4.5). This percentage suggests that the athletes were ill fairly frequently. However, it is important to realize that this account was very subjective in nature, and that the illnesses were not necessarily clinically diagnosed by a medical doctor. Some of the subjects may have reported that they were ill if they had a minor headache, while other subjects may not have stated that they were ill unless they had more severe

symptoms (e.g. flu, cold, upper respiratory tract infection). In future research, it would be beneficial to clarify what constitutes illness to the subjects (e.g., doctor diagnosed illness). This information will be revisited when examining the immune function measures. Mackinnnon (1997) has suggested that cumulative and possibly chronic alterations in immune function due to daily intensive exercise are responsible for the high rate of infectious illnesses among athletes.

3. Frequency of Menstruation:

The information that was obtained regarding the frequency of menstruation for the female subjects was of limited value. Each of the female subjects recorded the days when they were training when they had their period. However, on the days when the subjects did not train, no information was provided, and therefore the length of the period had to be estimated (e.g., for cycles that ended on a weekend). Based on the data presented in Table 4.6, it is clear that none of the subjects were amenorrehic. However, when an examination was done of the number of days between cycles for each of the female subjects, it became clear that several of the athletes had irregular menstrual cycles. According to Kreider et al (1998), the irregular menstrual cycles seen in active and athletic women may be due to periods of energy deficiency. Female athletes may have increased energy expenditure due to the physical and psychological stresses of training. As well, restricted eating practices may lead to a kilocalorie deficit, which can influence the secretion of reproduction hormones in female athletes. As was previously mentioned when discussing the results from the dietary records, the female subjects took in significantly less kilocalories during the crash period compared to the build period (2559.2 Kcal versus 3200.5 Kcal respectively).

This implies that they were not meeting the energy requirements for the level of training that they were undertaking. Although it is beyond the scope of this study, it would be interesting to examine the subjects who presented with irregular menstrual cycles and their dietary records to determine whether they were at a greater risk of being overtrained.

D *HEMATOLOGICAL MEASURES:*

1. Red Blood Cell Markers:

The results for the red blood cell markers demonstrated that there was a significant main effect of time for the red blood cell count, mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC), but not for hemoglobin or hematocrit (see Table 4.7).

The RBC count decreased from the baseline value with training. This finding is supported by Schumacher et al (2002), who found that endurance trained male athletes (n=426) had lower red blood cell counts compared to sedentary male individuals (n=104). Several authors have suggested that the reduced RBC count is due to exercise-induced plasma volume expansion (Schumacher et al; Shaskey & Green, 2000).

The mean RBC count was lowest during the crash period in the current study. Shaskey & Green (2000) have suggested that the stress of exhaustive exercise or very hard training causes the greatest plasma volume increase. Specifically, plasma volume expansion may be 6% to 25% greater than baseline following hard endurance training (Shaskey & Green). Unfortunately, plasma volume was not measured in this study to determine if this was in fact what occurred.

Although not significant, the results for Hb during the taper period were interesting, and therefore notable to mention. During the taper, the mean Hb concentration of the group increased to 150.4 g/L, from 148.8 g/L during the crash period (see Figure 4.5). This is in line with the significant increase in RBC count during the taper compared to the build and crash tests (see Figure 4.6). According to Houmard & Anderson Johns (1994), a restoration of Hb levels has been observed in competitive swimmers following various tapers that involve a reduction in training volume. This is important, as an increase in Hb concentration prior to competition may enhance oxygen carrying capacity and therefore physical performance. The elevation in Hb levels may be associated with a decrease in exercise-induced hemolysis from a reduction in training volume (Houmard & Anderson Johns).

Significant changes across time were also found for the group's mean cell volume (MCV). Specifically, the MCV significantly increased from the baseline value with training (see Figure 4.8). Schumacher et al (2002), suggested that endurance athletes experience increased turnover of red blood cells with accelerated destruction of these cells by different exercise-related mechanisms (e.g. squeezing and rupture of erythrocytes during muscle contraction). Schumacher et al also demonstrated a shift in the erythroid blood profile of endurance trained athletes toward younger cells with higher mean cell volumes. This corresponds with the results of this study. According to Mackinnon et al (1997), an increase in MCV is beneficial, as younger red cells appear to be more efficient in O_2 transport. Specifically, younger cells are more deformable, which reduces blood viscosity and contributes to enhanced cardiac output and blood flow during exercise.

Further, significant changes were observed in this study for the mean cell hemoglobin concentration (MCHC). The results demonstrated that the highest MCHC occurred during the crash period (see Figure 4.9). The MCHC is an expression of the average concentration of Hb in the red blood cells, and as such, gives the ratio of the weight of the Hb to the volume of red blood cells (Fischbach, 1996). An increased MCHC value signifies that a unit volume of packed red blood cells contain more hemoglobin than normal. Therefore, the increase observed in this study during the crash period may be a positive adaptation to intensive training.

2. Iron, Total Iron Binding Capacity and Ferritin:

The results from this study demonstrated that there was a significant main effect of time for iron, total iron binding capacity (TIBC) and also for ferritin. However, only the results for iron showed significant differences between specific testing periods (as revealed through *post hoc* analysis).

In general, the mean iron concentration was the highest at the baseline test (20.4 umol/L) (Figure 4.10). According to Schumacher et al (2002), iron and especially ferritin levels have been demonstrated to be reduced in athletes, due to higher iron turnover and increased synthesis of iron-containing proteins. As well, in athletes there is an increased loss of iron through sweat, the intestines and the kidneys. Our data supports this statement, as the iron levels were significantly reduced once the subjects started training. Similarly, the ferritin levels, although not statistically significant, were also reduced with training (compared to baseline values) (see Table 4.8). Nevertheless, both the iron and ferritin levels were still within the normal reference range as outlined by the Dynacare

Kasper medical laboratory in Edmonton (see Table 2.3). However, in this study, the decrease with training in iron and ferritin levels (compared to baseline values) may indicate that the iron loss (resulting from various causes including iron uptake, iron redistribution, and iron consumption) was incompletely matched by iron intake. These results, along with the results from Rietjens et al (2002) who found similar results in 7 male and 4 female elite tri-athletes, suggests that reasonable iron supplementation during periods of severe training should be considered.

E) IMMUNOLOGICAL MEASURES:

1. White Blood Cells and Differential:

The results of this study demonstrate that white blood cells (leukocytes), neutrophils, lymphocytes, monocytes and eosinophils did not change significantly across the training season. These results coincide with the review of literature presented by Nieman & Klarlund (2000), which suggested that there are few chronic effects of exercise training on immune cell numbers, and that clinically normal levels are observed in most athletes.

Basophils, on the other hand, did increase significantly with time (see Table 4.9, Figure 4.13). The function of basophils is to release histamine and other chemicals that decrease inflammation in damaged tissues (Martini & Bartholomew, 1997). Therefore, basophil counts are used to study allergic reactions (Fischbach, 1996). In terms of our results, although an increase in basophils was observed throughout the training season, the values were still well within the normal reference range of 0.0-0.3 x 10^9/L (Dynacare Kasper Medical Laboratory, 2003). Therefore, the changes that were observed were not clinically significant and therefore were unlikely to be of any real physiological significance.

However, it may be postulated that the increase of basophils may have helped to decrease inflammation of damaged tissue due to training.

2. T-cells and Natural Killer Cells:

The results from this study demonstrate that there was a significant change in total CD4+ (helper) T-cells across testing periods. As well, the CD4+/CD8+ ratio changed significantly with time (Table 4.10). In terms of the CD4+ (helper) T-cells, the percentage was lowest during the baseline test period (50%) and increased with each test, reaching its highest value at the crash test period (59%) (see Figure 4.19). The ratio of CD4+/CD8+ also followed a similar pattern. At the baseline period, the CD4+/CD8+ ratio was 1.9, at the build test the ratio was 2.0, and then at the crash test the ratio was 2.3 (see Figure 4.21). These results are in contrast to Gleeson et al (1995) who reported no changes in CD4+, CD8+ and the CD4+/CD8+ ratio over a 7-month season in elite competitive swimmers (15 males and 11 females aged 16-24 years). According to Kreider et al (1998), several other studies have also reported no changes in lymphocyte counts or sub-populations after short term intensified training or between periods of low and high intensity training. In terms of this study, it may be speculated that the increase in CD4+/CD8+ ratio signifies increasing immunocompetence for the athletes as a whole. This assumption is made based on the fact that previous research has suggested that a decreasing ratio (below 1.5) is a potential indicator of immunosuppression (Castell & Newsholme, 2001).

Also in contrast to other research, this study did not find any significant changes in the NK cells with time. However, although the changes were not statistically significant, they may still provide insight into the effects of training on NK cells. In this study, the

percentage of NK cells (CD16+CD56+) decreased from the baseline through to the crash test period (see Figure 4.22). Several researchers have observed a decrease in NK cells with training (Mackinnon, 2000; Gleeson, 2000; Gleeson et al, 1995). For instance, Gleeson et al (1995) found NK cell numbers declined by 30% -40% after 7 months of intense swim training. Unfortunately, the biological significance of a decrease in NK cell numbers has not yet been determined, but it is speculated that a fall in NK cells may leave an athlete susceptible to viral infections and illnesses (Gleeson et al, 2000). According to Mackinnon, elite athletes, frequently training twice per day (such as the athletes involved in this study), may require an extended period of time in order for their NK cells to recover following each session of intense exercise. As is evident, the results for NK cells are contradictory to the results for the CD4+/CD8+ ratio.

3. Salivary Immunoglobin A:

The results of this study demonstrate that S-IgA levels did not change significantly with different phases of training, likely due to individual variability (as evidenced by the range of scores) (see Table 4.11). Mackinnon & Hooper (1994) also reported no change in S-IgA levels in 14 elite competitive swimmers over a 6 month training season leading up to a major competition. These results, however, are in contrast to the majority of other longitudinal studies that have examined the impact of long term intensive training on mucosal immunity. The most frequent consensus among other studies is that S-IgA concentrations will decrease over long-term training periods (Gleeson, 2000; Krzywkowski, Petersen, Ostrowski et al, 2001; Gleeson et al, 1995).

4. Glutamine and Glutamate:

The group results for plasma glutamine and glutamate revealed that there was a significant decrease in glutamine concentrations across the different phases of training (Figure 4.24), but not in glutamate. Further, a significant main effect of time was also observed for the glutamine: glutamate ratio.

Rowbottom et al (1996), suggested in a review article that changes in glutamine concentrations across a training season were dependent upon whether the training was balanced or not. The review suggested that with balanced training (wherein exercise and recovery are undertaken in a sufficiently balanced ratio), glutamine levels should be increased with training. For example, Rowbottom et al found a significant increase in plasma glutamine levels in 8 triathletes (gender and ages unknown) over a 10 month training season. These athletes were considered optimally trained as significant performance improvements were seen across the training season (Rowbottom et al). Smith & Norris (2000) also suggested that an elevated plasma glutamine concentration in an athlete represents a positive adaptation to a well-balanced training program.

On the other hand, several studies have shown that plasma glutamine levels decrease following prolonged or intensive training sessions (Nieman & Klarlund, 2000). For these studies, the athletes were subjected to a period of overload training. For example, Smith & Norris (2000) found glutamine levels to decrease with heavy training in 52 national team athletes (31 males and 21 females). The decrease in glutamine is likely due to a net over-utilization by the liver, kidneys or cells of the immune system (Hiscock et al, 2002). The decreases in plasma glutamine following exercise stress may be sufficient to reduce

immune reactivity and to increase an athlete's susceptibility to infection (Rowbottom et al, 1996).

Therefore, the results of this study may suggest that the athletes did not have enough time to recover from the overload training prior to their major competition. Rowbottom et al (1996), suggest that overload training has a cumulative effect in terms of decreases in plasma glutamine level, such that prolonged periods of time are required for glutamine levels to recover fully.

Furthermore, the results of this study demonstrate a significant change in the glutamine:glutamate ratio across the different phases of training. Specifically, the glutamine:glutamate ratio remained fairly constant for the baseline (mean =12.2), build (mean = 12.7) and crash (mean = 12.8) test periods, but decreased significantly for the taper test (mean =9.2) (see Figure 4.25). According to Smith & Norris (2000), the ratio of glutamine to glutamate represents an athlete's overall tolerance to training. These authors found that the glutamine:glutamate ratio decreased significantly during heavy training. Smith & Norris also suggested that an increased glutamine:glutamate ratio should be observed when athletes are in a tapered condition (or when the training volume is low). This is in contrast to the results of this study, which found the ratio to decrease with the taper. This would suggest that the athletes in this study were not at an optimal level to perform immediately prior to their major competition. This may be due to an ineffective taper, or to a carry-over of fatigue from previous phases of training.

F) *HORMONAL MEASURE*

1. Cortisol:

The results for cortisol are very difficult to interpret. Despite careful attempts to standardize techniques for measuring cortisol, there was considerable interindividual and intraindividual variation in values for the various test periods as well as for the different times of day (morning, afternoon and evening). Further, there were several missing samples that added to the difficulty in interpretation. A decision was made to examine the morning cortisol results of the 14 subjects, and the results from the 3 subjects who completed all of the cortisol tests.

In terms of the morning cortisol for all 14 subjects, a decrease in mean concentration was observed with each testing period (35.7 nmol/L, 33.4 nmol/L, 26.9 nmol/L, 18.6 nmol/L from baseline, build, crash and taper tests respectively) (Table 4.13).

The results from the 3 subjects who provided all of their cortisol samples demonstrated a similar picture. In the morning, the average cortisol concentration for these 3 subjects decreased with each testing period, reaching its lowest values during the taper period. Similarly, the average afternoon and evening results also showed that cortisol was lowest during the taper (Table 4.14).

The finding that cortisol concentration decreased significantly (at all times of day) during the taper was very interesting. Studies that have examined the cortisol response to tapering have produced discrepant findings. For instance, Mujika et al (1996b) examined 8 highly trained male swimmers (mean age 21.1 years), and found that cortisol levels did not change significantly with a taper. Conversely, Bonifazi et al (2000), examined 2 groups of male swimmers (group 1: n=8, aged 19-25 years; group 2: n=10, aged 18-22 years), and found

cortisol levels to decrease significantly with tapering. Bonifazi et al suggested that a decrease in cortisol levels was a prerequisite for improved performance, and that a decrease in cortisol at the end of a training period could be considered as an index of adaptation to the work load. In this study, the overall trend of a decrease in cortisol during the taper may suggest that some of the athletes were exhibiting a positive adaptation to training. However, it would appear from other markers that many of the subjects had not in fact adapted positively to training prior to the major competition. The individual results for cortisol during the taper period are widely varying. This is a limitation of reporting mean results.

The results for the crash period were very surprising and unexplainable. This study hypothesized that the cortisol concentration would increase during overload training (e.g. during the crash period). Previous research has shown resting cortisol levels to increase with overload training (Kirwan et al, 1988; Costill et al, 1991; Bonifazi et al, 2000) or to remain unchanged (Mujika et al, 1996b). Researchers who have demonstrated an increase in cortisol levels with hard training have suggested that there is decreased pituitary sensitivity to cortisol negative feedback (Bonifazi et al; Duclos, Corcuff, Arsac et al, 1998). As well, the anti-inflammatory role of cortisol may explain the elevated concentrations of this hormone during hard training (e.g., there would likely be increased muscle damage during periods of hard training) (Kirwan et al). Unfortunately, this does not explain the results of this study. It is unclear why a decrease was observed during the crash period, but it is possible that the choice of specimen used for cortisol determination in this study may partially explain these findings.

In this study, saliva was the specimen of choice for the cortisol determination. While saliva is a stress-free, non-invasive, and practical way of measuring cortisol (Duclos, Corcuff & Arsac et al, 1998), there are some concerns that need to be addressed. Firstly, it is important to realize that because only the free fraction of the hormone enters the salivary glands, the concentration is 50- to 100- fold lower in saliva compared to serum or plasma (Tremblay & Chu, 1994). As was evidenced in this study, the salivary cortisol concentrations were often so low in the afternoon or evening that they were reported as undetectable. Further, Tremblay & Chu advise caution in the use of saliva testing due to the fact that contamination with traces of blood (due to lacerations in the gum and mucosa) would falsely increase the salivary hormone concentration.

However, despite the concerns regarding the use of saliva, the results of the 3 subjects who completed all of the cortisol samples did demonstrate the expected diurnal variation of cortisol throughout the day. Evening cortisol values were about half of the morning values. Previous studies have shown that cortisol concentration is at its lowest near midnight, with its highest values occurring between 8 AM and 10 AM (Tremblay & Chu, 1994). This pattern was clearly evidenced in this study, and can be observed in Figure 4.27. Cortisol concentrations were normally highest during the morning (6-8AM) tests, decreased significantly by the time the afternoon (4-5PM) sample was given, and then decreased again for the evening (8-10PM) samples.

To conclude, this discussion has demonstrated that it would be very difficult to place great reliance on the cortisol information that was obtained for any single individual, other than the fact that diurnal variation is evidenced. Although previous research has shown that cortisol concentrations can reflect long term training stresses, this study did

not display the expected change with overload training. Further, the RIA used was relatively expensive and time-consuming. Therefore, based on this study's results, it appears as though salivary cortisol has limited value for the monitoring of training status in these athletes.

G) *CARDIOVASCULAR MEASURES:*

The subjects involved in this study were required to perform the dynamic postural heart rate (Rusko) test as well as the spectral heart analysis at each of the test periods as a means of assessing heart rate variability. Previous research has suggested that heart rate variability analysis is an appropriate tool for diagnosing training loads and appraising cumulated fatigue (Pichot et al, 2002). Surprisingly, none of the results from either of these two tests were statistically significant across time.

For the dynamic postural heart rate (Rusko) test, two numerical values were calculated. As a reminder, the protocol for the test involved lying for 8 minutes and then standing up for 2 minutes, with heart rate measurements taken every 5 seconds. Based on the heart rate data obtained, the first calculation that was made was the average heart rate for the last 30 seconds of standing. For this study, the average heart rate was 89.3 bpm at the baseline period, 82.0 bpm at the build test, 81.7 bpm at the crash test, and 86.5 bpm at the taper test (see Figure 4.29). It is surprising that the lowest mean heart rate was observed during the crash period. This study had hypothesized that an increase in the standing heart rate would be observed with overload training (i.e. the crash period). During the crash period, it was anticipated that the subjects in this study would be experiencing fatigue. Previous research has demonstrated that sustained tachycardia during the later

phases of standing would indicate that sympathetic stimulation had been maintained (Neto et al, 1980), which has been identified as a sign of impending fatigue (Iellamo et al, 2002). Norris et al (2001) also speculate that an elevated heart rate (i.e. 8-10 beats + above normal) during the last 30 seconds of the test is a sign of some failure to recover adequately (SNC coaches seminar, unpublished). They suggest that this may be due to hard training and not having sufficient recovery time, the onset of illness, or other forms of life stessors. This leads to the conclusion that the athletes in this study as a group may have been adapting adequately to the increased training volume during the crash period as they did not experience an increase in standing heart rate.

Despite this, it is interesting to note that the average heart rate of the last 30 seconds of the test increased again with the taper. Perhaps this suggests that there was not enough recovery time between the overload period (i.e. the crash test) and the taper. While this finding was not statistically significant, it may be physiologically significant for the athletes.

The second numerical value that was derived from the dynamic postural heart rate test was a Rusko score. As can be seen from Figure 4.29, the pattern across time for the Rusko score is very similar to the pattern for the average heart rate for the last 30 seconds of standing. Therefore, it is believed that the Rusko score could be used as an alternative to the average heart rate of the last 30 seconds of standing. However, the Rusko score may in fact be more desirable, as it also takes into account the heart rate response to lying. As a result, the Rusko score is a means of examining orthostasis, or assuming an upright position. However, more research is needed in this area.

The results for the spectral heart rate analysis also failed to show a significant main effect of time. The results from the spectral heart rate analysis were used to make several calculations. The first calculation involved developing a mathematical score for total heart rate variability based upon a linearly weighted interpretation. According to Norris (2003, personal communication), a lower score is more desirable for an athlete. On the other hand, a maximum score of 27 can be achieved, but this would imply that an individual's cardiac system is not adapting to the demands of the exercise training. This would be a cause for concern. In this study, the total heart rate variability score remained fairly constant across the testing sessions. The total heart rate variability scores were 15.6, 15.0, 16.0, and 16.0 during the baseline, build, crash and taper times, respectively (Table 4.16).

The OmegaWave Sport Technology System, which was the instrument used to perform the spectral heart rate analysis, was also able to provide information regarding the percentage of the very low frequency, low frequency and high frequency modulators of heart rate. Research has shown that the high frequency modulation of heart rate reflects parasympathetic activity (Hedelin et al 2000a; Sato et al, 1995). The low frequency and very low frequency modulators of heart rate are more difficult to interpret. Researchers are unsure as to what the very low frequency component entails, but the low frequency component is believed to reflect both sympathetic and parasympathetic activity (Hedelin et al; Sato et al). As a result, the signs of an adaptation in sympathetic activity on its own are difficult to interpret. Therefore, this study will focus on the high frequency component. At the baseline period, the group's mean high frequency percentage was 55.7%, but at the build period it had dropped to 45.2%. At the time of the crash test, the high frequency

percentage was 57.4%, and at the taper test the percentage was 60.2% (see Figure 4.30). It is interesting that the lowest high frequency percentage was observed during the build period. Perhaps the initial impact of training after a period of rest during the off-season caused a slight shift towards more sympathetic modulation (which was not of statistical significance). The reason for this shift, however, can not be explained at the present time.

All in all, based on the lack of statistical significance for all of the heart rate variability measures, it may be concluded that endurance training does not, in itself, result in a significant adaptation of the autonomic nervous system. It is likely that the lack of statistical significance was due to the inter-individual variability in the results, as can be evidenced by the range of scores (see Table 4.16). Therefore, the use of heart rate variability may be more useful for examining an individual's response to training as opposed to the group as a whole.

H) PERFORMANCE MEASURES:

The athletes involved in this study participated in three swimming specific test sets at each of the test sessions: a 4 x 50m test set, a 5 x 200m test set, and a 100m time trial.

For the purpose of this study, it was hypothesized that the 4 x 50m test set (predicted 100m time) would accurately reflect performance in the 100m time trial. As can be observed in Figure 4.33, the 100m time trial and the predicted 100m time followed a similar pattern across test periods. For both of the tests, the baseline period proved to be the slowest for the majority of the swimmers. By the time of the build test, the athletes had improved their speed, and by the crash period the subjects were swimming their fastest for these tests. Interestingly, at the taper test, the times for these tests were

slightly slower than at the crash period. This finding was concerning, as a taper should result in improved performance. According to Houmard & Anderson Johns (1994), a 3% improvement in performance can be anticipated with a properly designed taper. In this study, it is possible that the athletes chose not to perform the tests to the best of their ability due to fear that it might jeopardize their taper. Analysis of the RESTQ questionnaire, which is to be done at a later date, may provide support for this suggestion. However, at the current time, these results suggest that the taper may not have been designed adequately.

Further, it is interesting that the predicted 100m time and the 100m time trial followed the same pattern across the testing sessions. As can be seen from Figure 4.33, however, the predicted 100m time was always faster than the actual 100m time trail. Therefore, coaches who utilize the 4 x 50m test should take this information into consideration.

The results for the 5 x 200m test set, on the other hand, did not demonstrate a significant change across the testing periods. As discussed, the heart rate/velocity graph that was developed for each athlete at each testing session was used to develop a predicted 400m time. From examining the results for the predicted 400m time (Table 4.18), it can be seen that the times remained fairly constant across the build (mean =265.7 sec), crash (mean=264.6 sec) and taper (mean =264.4 sec) periods (see Figure 4.34). Similarly, the mean slope graph of the group's heart rate and velocity for the 5 x 200m test set did not change significantly across test periods (see Figure 4.35). This implies that at a certain swimming speed, the athletes had a fairly consistent heart rate across the training season.

Taken together, this suggests that the subjects' aerobic fitness did not change dramatically from the time of the build period in October to the taper in November.

Further, the predicted 400m time during the taper period was compared to the actual 400m freestyle performance of 7 of the subjects at their major competition. This was done in order to assess the validity of the 5 x 200m test at predicting a 400m time. Appendix K (Table 1A) displays the times of the 7 subjects who swam 400m freestyle at the major competition, their predicted 400m times, as well as the difference in time between the actual and predicted 400m freestyle. It can be noted that for 4 of the subjects, the predicted 400m time under-predicted their actual performance. For the other 3 subjects, the predicted 400m time over-predicted what they actually accomplished at the competition. Taken all together, the predicted 400m time was on average 4.3 seconds faster than the subjects' actual 400m performance at the swim meet. This average would suggest that the 5 x 200m test was relatively effective at predicting 400m performance. However, the range of time differences between the predicted time and the actual time would suggest otherwise. For these 7 subjects, the time difference ranged from -14.8 seconds to +18.6 seconds, which is a substantial amount of time in a 400m freestyle. Appendix K (Table 1B) displays the specific data from the 5 x 200m test during the taper period including the slope of the heart rate velocity graph, the r^2 value, the predicted 100m time at heart rate max, as well as the predicted 400m time. From this information, it appears as though all of the subjects performed the 5 x 200m test according to protocol except for subject 07. This one individual did a poor job of descending the 200's and as a result his r^2 value was only 0.84. Interestingly, this subject's predicted 400m time was only 1 second slower than his actual 400m performance at the competition. Therefore, more

testing needs to be performed with a greater number of swimmers in order to determine the accuracy of the 5 x 200m test at predicting 400m performance in competition.

It is important here to note and recognize the limitation of the 5 x 200m test set as it was used in this study. Due to time and cost restrictions, this study did not perform tests (e.g., VO_2 max tests) to determine each subject's maximum heart rate. Rather, subjects simply provided the primary researcher with their highest heart rate that they had recorded during intensive training or after races. The subjects frequently monitor their heart rates during training and competitions, and therefore are very knowledgeable in this regard. However, it is important to realize that if the maximum heart rate was incorrect, the calculation of the predicted 400m time would be faulty. This would especially hold true if an athlete was experiencing a blunted maximum heart rate during a particular stage of training (i.e. the predicted 400m time would be overestimated).

To conclude this discussion on the group results, the subjects' performance at their major competition must also be examined. Table 4.19 displays a percentage of best times for each athlete as determined from their 3 main swimming events. Based on these results, an average of performance at the major competition compared to previous best times for the 14 athletes together was determined to be 98.6%, with a range of 93.7% to 100.9%. Specifically, 3 swimmers achieved 100%+ best times, 8 swimmers achieved between 97-99% best times, and 3 swimmers performed at less than 97% of their best. According to Norris (2003, personal communication), competitive swimmers should be able to perform to a level which is 97-98% of their best times at in-season (non-tapered) competitions. Therefore, at a competition where athletes are tapered, the performance levels should be higher. Since only 3 athletes in this study achieved 100%+ best times, it may be concluded that the taper

may not have been as effective as it should have been. This assumption is supported by the results for the other performance measures, which also suggest that the taper was not completely effective.

DISCUSSION: RESPONDERS VERSUS NON-RESPONDERS

The previous discussion regarding the group results as a whole provided insight into the physiological changes that took place in the subjects across the different phases of the training season (baseline, build, crash and taper periods). Based on the discussion, however, it is difficult to delineate the normal changes that occurred with training from the abnormal changes that may be associated with overtraining. According to Hopper & Mackinnon (1995), the difficulty of differentiating normal from abnormal physiological responses to training presents a major problem for researchers, especially when changes in physiological measures are in the same direction as those expected with training. Therefore, the goal of this next section is to differentiate between the individuals who performed 100%+ best times at the major competition (RESPONDERS, n=3) and the subjects who performed poorly (e.g. less than 97% best times) (NON-RESPONDERS, n=3). For the purpose of this study, it is speculated that the non-responders were overreached or overtrained. Previous researchers have suggested that stagnancy in performance is sufficient to indicate overtraining when considered in conjunction with other signs and symptoms (Hopper & Mackinnon). Therefore, this next section will examine all of the study variables for the responders and non-responders. Since the sample size of the responders and non-responders was too small to run repeated measures ANOVAs, a comparison of the two groups was made based on descriptive statistics (e.g. Figures and Tables).

A) SUBJECT CHARACTERISTICS: RESPONDERS VS. NON-RESPONDERS:

The 3 responders (aged 16, 18 and 18 years) and the 3 non-responders (aged 16, 16 and 18 years) were all females. This was beneficial as gender can influence a number of the physiological markers that were studied. Further, the fact that the responders and non-responders were relatively close in age was advantageous. As a result, gender and age could be eliminated as confounding variables in this discussion. As well, the training style was similar for both groups. Of the non-responders, 2 were distance swimmers, and 1 was a middle distance swimmer, and of the responders, 1 was a distance swimmer and 2 were middle distance swimmers.

In terms of weight, waist circumference, and body mass index (BMI), the measurements for the responders and the non-responders did not seem to change dramatically across the test sessions. Interestingly enough though, the responders recorded their lowest weight, waist circumference and BMI values at the start of the season (during baseline testing), whereas the non-responders achieved their lowest values during the taper period (see Table 4.21). Therefore, it is speculated that the non-responders were not meeting the energy demands of their training program. The results for their dietary records, which are discussed next, support this speculation.

B) ENERGY INTAKE: RESPONDERS VS. NON-RESPONDERS:

Research has suggested that nutrition plays an important role in the prevention of athletic fatigue (Sherman & Maglischo, 1991). There is also an abundance of evidence to suggest that nutritional deficiencies alter immunocompetence and increase the risk of infection (Gleeson et al, 2000). In this study, the non-responders consumed less total

calories, including less grams of protein, carbohydrate and fat during the 2nd dietary recording (crash period) compared to the initial assessment which was done during the build period. On the other hand, the responders consumed relatively the same number of calories at each test, and especially consumed more fat during the crash period. However, similar to the non-responders, the responders consumed less protein and carbohydrate during the 2nd dietary recording.

Likely of most significance is the fact that the non-responders consumed less calories during the 2nd dietary record (crash period) compared to the 1st record (build period) (see Table 4.22). This, along with the knowledge that the non-responders recorded their lowest weight, waist circumference and BMI values during the taper period, would suggest that the non-responders were not meeting their energy demands for training. According to Venkatraman & Pendergast (2002), the diets of athletes should be balanced so that total caloric intake equals energy expenditure, and so that carbohydrates and fats utilized during exercise are replenished. Achieving energy balance is essential for the maintenance of lean tissue mass, reproductive and immune function, and optimal athletic performance. If energy intake is inadequate, such as in the case of the non-responders, fat and lean tissue mass will be used by the body for fuel, which will result in a loss of strength and endurance (American Dietetic Association & Dieticians of Canada, 2000). Therefore, meeting energy needs should be a priority for athletes.

C) *TRAINING: RESPONDERS VS. NON-RESPONDERS:*

Based on the results described in chapter 4, the non-responders swam more Km/week, especially during weeks 3, 5 and over the last 4 weeks of the study, compared to

the responders (see Table 4.23). The greatest difference was noted during week 7 (non-responders = 56.1 Km, responders = 30.0 Km). Further, the non-responders completed more volume (both swimming and dryland training) during the taper period compared to the responders, even though one of the non-responders trained infrequently during the taper period due to illness. It appears probable that the higher training volumes the non-responders were performing, at various points during the study, were too great for them to handle. This assumption is made based in part on the fact that the non-responders reported lower training intensities compared to the responders throughout the majority of the study. Although there is some controversy regarding whether overtraining is induced more through increased intensity or increased volume, the results from this study would suggest that the increased training volume was what led to the performance decrements in the non-responders at the major competition. This finding is in accordance with the study performed by Lehmann et al (1992), which examined 8 male middle distance and 9 male long distance runners (mean age 34 years). In their study, an increase in training volume caused a considerable decrease in maximal performance, with performance incompetence for months, whereas an increase in training intensity not only failed to cause an overtraining syndrome, but the subjects demonstrated an increase in endurance, and a general improvement in performance.

In relation to the subjective ratings of training fatigue, the non-responders appeared to experience higher levels of fatigue throughout the majority of the study. Figure 4.39 illustrates that the non-responders had a significantly higher fatigue level during weeks 5, 7, 8 and 10. This finding of increased fatigue level may be of relevance to coaches, as it could be used to indicate that swimmers need to back off of training. As well,

the higher fatigue rating during the taper is an indication that the athletes were not ready to perform optimally at the major competition. Perhaps an athlete's weekly fatigue rating of 5 or above (on the 7 point scale) could be used by coaches as a warning sign.

The training log books of the responders and non-responders also provided valuable information regarding the frequency of illnesses of the subjects. As was mentioned in chapter 4, the responders were ill for 13.7% of the workouts, whereas the non-responders stated that they were ill at 22.3% of the practices. According to Nieman & Klarlund (2000), symptoms of upper respiratory tract illness (URTI) (e.g. runny nose, nasal congestion, sore throat) are often reported by endurance athletes who undergo high volume training. As well, Nieman & Klarlund suggest that the incidence of URTI is related to training volume. Specifically, a 'J-curve' model has been proposed to describe the relationship between exercise volume and susceptibility to URTI; moderate exercise may reduce susceptibility to illness, but the risk is progressively elevated by increasingly intense exercise. This 'J-curve' model may explain the higher incidence of illness in the non-responders compared to the responders in this study.

D) *HEMATOLOGICAL MEASURES: RESPONDERS VS NON-RESPONDERS*

In this study the non-responders had lower hemoglobin, red blood cell count, hematocrit and mean cell hemoglobin concentrations (MCHC) at each of the test sessions compared to the responders (see Table 4.25). Further, the non-responders experienced significantly lowered iron levels at the crash and taper periods, and had ferritin levels that were lower at each of the testing sessions (especially during the baseline and build periods) compared to the responders (see Table 4.26). According to Fischbach (1996), the term

"anemia" is used to describe a condition in which there is a reduction in the number of red blood cells, the amount of hemoglobin, or the volume of packed cells (hematocrit), or any combination thereof. Typical signs of iron deficiency anemia include reduced serum iron (e.g. 5-10umol/L), and greatly decreased serum ferritin (e.g. >10ug/L) (Shaskey & Green, 2000; Higgins, 2000). Although the non-responders would not be clinically diagnosed as anemic, they did appear to have a form of iron deficiency, especially during the crash period (mean iron=8.3umol/L and mean ferritin = 26.7ug/L). This suggests that during the crash period the non-responders experienced an increased demand for iron. This statement is also supported by the fact that two of the non-responders had iron levels that were below the normal female reference range during the crash period, and one of these girls also recorded very low iron levels during the taper period (Dynacare Kasper Medical Laboratory, 2002). Conversely, none of the responders experienced low iron levels during the crash or taper periods. In fact, one of the responders experienced very high iron levels (i.e. above the reference range) during the baseline, crash and taper tests.

For the non-responders, the lower iron levels could have had a major impact on their performance. Iron is an essential constituent of hemoglobin, myoglobin, and several enzymes in the metabolic pathways (e.g., cytochromes of the electron transport chain) (Williams, 2002). Specifically, the ability of hemoglobin to combine with oxygen and to transport it is related to its iron component. Similarly, myoglobin requires iron to perform its roles of storing oxygen within muscle, and in facilitating the diffusion of oxygen from blood to mitochondria within the muscle cell (Foss & Keteyian, 1998). Therefore, iron is necessary for oxygen transport. As well, iron is critical to the process of electron transport leading to ATP resynthesis. In the electron transport chain, the hydrogens and

electrons are passed downward from a level of higher energy to a level of lower energy through reversible changes in the state of iron between Fe^{2+} and Fe^{3+}. It is this energy that is eventually used to resynthesize ADP and Pi back to ATP. Therefore, it is no wonder that people with iron deficiencies have little energy (Foss & Keteyian). Based on this information, it makes sense that lower iron levels would be detrimental to the athletic performance of the non-responders.

E) IMMUNOLOGICAL MEASURES: RESPONDERS VS. NON-RESPONDERS

1. White Blood Cells and Differential:

For the non-responders, the clinical significance of the results for the white blood cells and differential variables is not well understood. As evidenced in Figure 4.43, the non-responders experienced an elevated white blood cell count at the crash period, and then their lowest value at the taper period. The differential variables also appear to follow a similar trend (Figures 4.44 - 4.47). For the non-responders, the neutrophil, monocyte and eosonophil levels were all the highest during the crash period, but decreased with the taper. This was in contrast to the responders who maintained fairly consistent white blood cell count and differential values across each of the test periods. According to Higgins (2000), an increase in white blood cells is a protective response to injury, infection and inflammation, while a reduction in white blood cells leaves an individual at risk of serious, prolonged or chronic infections. These findings suggest that the non-responders experienced alterations in their immune function. This may explain why all 3 of the non-responders became ill either just before the major competition or immediately after.

2. T-cells and Natural Killer Cells:

When examining an individual's T-cells, researchers frequently utilize the CD4+ to CD8+ ratio. In this study, the CD4+ to CD8+ ratio did not appear to differ between the responders and non-responders. For both the responders and non-responders, the ratio values ranged from 2.0 -2.6 across the training season (see Table 4.28). It had been postulated prior to the study that the non-responders would experience a decreased ratio during the crash phase. This assumption was made based on the fact that previous research has demonstrated that a ratio below 1.5 is subnormal and may be a cause of or an indicator of immunosuppression (Castell & Newsholme, 2001). However, during the crash phase, the non-responders actually recorded their highest ratio value of 2.6. It would have been interesting to have seen what the ratio for the non-responders was during the taper period. It is unfortunate that a lab error occurred when analyzing the taper samples, as perhaps the ratio would have decreased at this time point. Therefore, more research is needed in this area.

The NK cells (CD16+CD56+) were also examined for the responders compared to the non-responders. During the crash period, the responders recorded their lowest CD16+CD56+ cell percentage, whereas the non-responders recorded their highest CD16+CD56+ cell percentage (see Figure 4.51). These findings are in complete contrast to previous research that has speculated that a fall in NK cell numbers may leave an athlete susceptible to viral infections and illnesses (Gleeson et al, 2000). With this belief in mind, it was anticipated that the responders would have a higher NK cell level throughout the training season, and the non-responders would experience the lower values. As was mentioned with the T-cell information, it would have been interesting to see how the values

changed with the taper. Perhaps a different picture would have been portrayed at that point.

3. Salivary Immunoglobin A:

Upper respiratory tract infections have been frequently related to the level of an athlete's S-IgA level (Krzywkowski, Petersen, Ostrowski et al, 2001; Nieman & Klarlund, 2000). Specifically, several researchers have suggested that a decrease in S-IgA levels may be associated with increased risk of upper respiratory tract infection (URTI) and/or overtraining (Krzywkowski, Petersen, Ostrowski et al; Gleeson et al, 1995). This is due to the fact that S-IgA plays a major role in immune protection at mucosal surfaces by providing specific antibodies in response to pathogens (Gleeson et al, 1999). In this study, the non-responders experienced 3 times lower S-IgA levels (71.0 ug/ml) compared to the responders (232.2 ug/ml) during the crash period (see Table 4.29). Therefore, this variable could help to explain why the non-responders became ill prior to or immediately after the major competition, while the responders did not.

4. Glutamine and Glutamate:

The comparison of the results for glutamine and glutamate for the responders and non-responders may prove useful for monitoring training. In this study, the responders experienced an increase in the glutamine:glutamate ratio during the crash period (15.4), whereas the non-responders experienced a decrease in the ratio (11.7) (compared to the baseline and build periods). According to Smith & Norris (2000), athletes who have developed a load tolerance to training, especially through aerobic work, tend to have higher

glutamine:glutamate ratios. This may explain why the responders had increased ratios during the crash periods, whereas the non-responders had decreased ratios.

Further, both the responders and non-responders demonstrated a decrease in the glutamine:glutamate ratio during the taper period. Specifically, the responders' ratio decreased to a value of 10.2, and the non-responders' ratio reached a low of 7.6. As was mentioned when discussing the group's glutamine and glutamate results, a decrease in the ratio is indicative of training that is not balanced (Smith & Norris, 2000). This suggests that the taper was not optimal for both the responders and non-responders. Despite this, it is important to realize that the low ratio value of 7.6 for the non-responders is more concerning than the value of 10.2 for the responders. According to Smith & Norris, athletes who have a potential for less tolerance to either increased volume or increased intensity of training load may be identified by relatively lower glutamine: glutamate ratios. Further, there is a "gray" area between managing the training load and overreaching. Therefore, in this study, it can be speculated that the lower glutamine:glutamate value for the non-responders may have placed these athletes in the "gray" area of overreaching, while the slightly higher ratio value for the responders indicated that they were still managing training. However, more research is needed in this area to confirm this speculation.

F) HORMONAL MEASURE: RESPONDERS VS. NON-RESPONDERS

The results for cortisol for the responders versus non-responders failed to show any clear relationship or pattern. Specifically, in terms of the morning cortisol, the results were completely inconsistent for the responders and non-responders (see Table 4.31). For example, when looking at the results for the responders, subject 02 achieved her highest

morning cortisol level during the taper period, and the lowest level occurred during the build period. This was in complete contrast to the 2 other responders, who both experienced their highest morning cortisol levels during the build period, and then their lowest levels during the taper period. In terms of the non-responders, the results were again quite varied. For example, two of the non-responders showed their highest morning cortisol levels during the baseline period, while the other non-responder showed the highest level during the crash period. For the non-responders however, there was a consistent finding in that the taper period was the lowest. The specific cortisol concentrations at each of the testing periods for the responders and non-responders can be found in Table 4.31. Further, this table illustrates the fact that numerous cortisol samples were missing for both the responders and non-responders during the afternoon and evening testing sessions. This also added to the difficulty in interpreting the results. Therefore it is difficult to draw any conclusions from these findings.

G) *CARDIOVASCULAR MEASURES: RESPONDERS VS. NON-RESPONDERS*

The first cardiovascular measure to be discussed is the dynamic postural heart rate (Rusko) test. Based on the data collected from this test, the average heart rate of each subject for the last 30 seconds of standing was calculated, as well as a Rusko score (see Table 4.33). When the results were examined for this test, a similar pattern emerged for both the responders and non-responders during the baseline, build and crash periods. However, at the taper test period, the Rusko score, and the average heart rate of the last 30 seconds of standing increased dramatically for the non-responders compared to the responders. According to observations by Norris et al (2001), an elevated heart rate (i.e. 8-

10 beats + above normal) may be a sign of impending fatigue. Based on the review of literature described in chapter 2, the majority of research in this area has demonstrated that sustained tachycardia during the later phases of standing would indicate that sympathetic stimulation has been maintained (Pichot et al, 2002; Uusitalo et al, 1998; Neto et al, 1980). Specifically, the literature suggests that very intensive training shifts the cardiovascular autonomic modulation from a parasympathetic toward a sympathetic predominance (Iellamo et al, 2002). In this study, it was anticipated that the non-responders would experience an elevated standing heart rate and Rusko score during the crash period. This was not observed for these athletes. Rather, the elevated Rusko score and increased standing heart rate were both observed during the taper period. These results suggest that there may have been an insufficient recovery period between the period of very hard training and the taper for the non-responders. This finding of an elevated Rusko score and increased standing heart rate may be of benefit to coaches and athletes. Despite the fact that more research is needed in this area, it is likely that the dynamic postural heart rate (Rusko) test would aid a coach in planning subsequent training if used on a regular basis. If a coach or athlete recognized an elevated Rusko score or an increased standing heart rate, adjustments in the volume and intensity of training could be made in order to optimize over-reaching and to avoid overtraining.

The results from the spectral heart rate analysis also appear to be beneficial for coaches and athletes in the monitoring of training. The results from the spectral heart rate analysis demonstrated that the non-responders experienced decreased parasympathetic modulation during the taper period, whereas the responders displayed an increase in parasympathetic modulation during the crash and taper periods (see Table 4.34).

This conclusion is made based on the fact that the non-responders showed a decrease in their high frequency component of heart rate variability (Figure 4.57), while the responders experienced an increase (Figure 4.56). Power spectral analysis has the ability to detect frequency specific oscillation in heart rate signals. Research has determined that the high frequency (0.15-0.45 Hz) modulation of heart rate reflects vagal activity (Hedelin et al, 2000a; Sato et al, 1995).

Furthermore, in this study, the results of the spectral analysis were used to develop a mathematical score for total heart rate variability. In this study, the responders achieved their lowest heart rate variability score (mean = 13) during the taper period, whereas the non-responders received their highest score (mean = 19) at this time period. A higher score would suggest that the athlete's cardiovascular system was not adapting to the demands of the exercise training. Therefore, the total heart rate variability score also suggests that the non-responders were unable to sufficiently recover from the crash period prior to the taper.

It is also important to mention that the OmegaWave Sport Technology System was able to provide specific information regarding the functioning of the athlete's cardiac system. For instance, the OmegaWave Sport Technology System reported for all of the responders during the taper test that their cardiac system was reasonably ready for any level of activity. On the other hand, the OmegaWave Sport Technology System produced a report for one of the non-responders (subject 14) during the taper test, that suggested that her cardiac system was not ready for activities of high volume, either high or moderate in intensity. For this subject, the system also reported that there were significant disturbances of heart rhythm, and that the athlete should consult a physician

for an ECG analysis. As another example, one of the other non-responders (subject 01) received a report from the system during her taper test that suggested that her cardiac system was not ready for activities of maximal volume and intensity. Therefore, it appears as though the OmegaWave Sport Technology System was able to fairly accurately predict performance.

Based on this discussion, it appears as though an examination of heart rate variability can provide useful information regarding the autonomic nervous system. Therefore, heart rate variability analysis may be of benefit to athletes and coaches in terms of monitoring the effects of training loads on performance.

H) PERFORMANCE MEASURES: RESPONDERS VS. NON-RESPONDERS

The results from the performance measures proved to be the most practical in nature. As could be expected, the non-responders were unable to perform optimally in either the 100m time trial or the 4 x 50m test set (i.e., the predicted 100m time). Therefore, coaches and athletes can use these two tests as a predictor of performance. Specifically, Figure 4.59 clearly demonstrates how the non-responders swam considerably slower in these two tests during the taper period compared to the crash period. In actuality, the non-responders' performance in these two test sets was almost the same as it was in the baseline period when they were not training. These tests provided a clear indication that the non-responders were fatigued prior to the major competition.

The results from the 5 x 200m test set also produced some interesting information. During the taper period, only 2 of the non-responders performed this test set. The other non-responder was already experiencing feelings of illness during the taper, and therefore

could not complete the 5 x 200 test during the taper period. Surprisingly, however, the 2 non-responders who did complete the test performed relatively well. For these two swimmers, the predicted 400m time from the taper test was the fastest they had recorded. As discussed above, the predicted 400m time was derived through extrapolation of the heart rate/ velocity graph for the 5 x 200 test to the subject's maximum heart rate. It is very likely however that maximum heart rate was blunted for these two swimmers during the taper test. This would cause the 5 x 200m test to over-predict their 400m performance. The actual results for both of the non-responders (subject 01 and subject 14) would appear to bear out this suggestion. Both are distance swimmers who competed in the 400m freestyle at their major competition. For subject 14, her predicted 400m time during the taper period was 264.8 seconds, but at the National Championships, she was only able to achieve a time of 277.9 seconds. Therefore, the 5 x 200m test over-predicted her time by 13.1 seconds. Similarly, subject 01 had a predicted 400m time of 276.0 seconds during the taper, but swam her 400m Freestyle in 290.6 seconds at the competition, an over-prediction of 14.6 seconds. Based on this information, it appears as though more research needs to be done in order to determine if the 5 x 200m test set is an appropriate means of predicting 400m performance. Unfortunately, the results from this study suggest that the 400m prediction is not as useful as the 100m prediction.

DISCUSSION: CASE STUDY SUBJECT 01

Subject 01 was examined individually because it was speculated that this athlete was overtrained. The term overtraining indicates that an individual has been stressed by training or extraneous factors to the extent that he or she is unable to perform at an

optimal level following a prolonged period of rest (i.e., greater than 2 weeks) (Fry et al, 1991a). Although this athlete was not clinically diagnosed as being overtrained, many of the variables measured in this study would suggest that she had reached a very high level of fatigue, which ultimately resulted in her poor performance at the Canadian National Championships. Specifically, the variables that are discussed for this subject include swim training volume, energy intake, iron, white blood cells, immunoglobin A, glutamine/glutamate, heart rate variability and the swimming test sets.

When first looking at the results for subject 01, it became clear immediately that her swim training volume was a matter of concern. This subject was a distance swimmer, so was used to high volumes of training. Unfortunately, the results from her log book demonstrated that her training volume was quite varied from week to week. In general, subject 01 had similar swim volumes compared to the group mean for weeks 1-6. However, beginning in week 7, she had a considerably higher volume of training with 70 Km swum and 195 minutes of dryland training. This was even more alarming considering that her swim volume in week 6 was only 29.1 Km with 110 minutes of dryland training. From her training log book, it appears as though she was ill during week 6. It is speculated that week 7 was where the problem began for subject 01. Further, Figures 4.62 and 4.63 demonstrate that subject 01 also had a significantly higher swim training volume and spent more time doing dryland activities during weeks 9 and 10 (the taper period) compared to the rest of the group. As was mentioned earlier in this chapter, a period of very hard training should be performed at least 4 weeks prior to a major competition (Norris, 2003, personal communication). Further, research has suggested that a reduction in training volume of 60% -90% should occur during a taper (Houmard & Anderson Johns, 1994). This clearly did

not happen for subject 01. Therefore, the results from this study would suggest that this athlete performed her block of very hard training too close to the major competition, and also that the volume performed during the taper period was too high.

In addition, the energy intake of subject 01 was a concern. Specifically, subject 01 consumed less calories at both testing periods compared to the average of the other female subjects. As well, subject 01's calories were lower during crash period compared to the build period (2190 vs. 2559 Kcal). Further, subject 01 recorded her lowest weight, waist circumference and BMI during the taper period. This data implies that subject 01 was not meeting the energy demands of training, which may have consequently affected her performance. According to the American Dietetic Association & Dieticians of Canada (2000), inadequate energy intake can lead to weight loss, disruption of reproductive function, immunosuppression, and a loss of muscle mass.

Next, an examination of subject 01's hematological variables revealed that her iron levels were very low especially during the crash and taper periods. Of utmost concern was the fact that her iron levels were below the normal female reference range during the crash and taper periods (Dynacare Kasper Medical Laboratory, 2002). Specifically, subject 01's iron levels reached a low of 4 umol/L during both the crash and taper periods. Figure 4.66 displays how subject 01's iron levels were significantly reduced during these periods especially compared to the group. Very low iron levels are concerning due to the fact that iron is essential for hemoglobin and myoglobin function, as oxygen forms a weak link with the iron contained within the heme molecule (Higgins, 2000). Further, iron is critical to the process of electron transport leading to ATP resynthesis (Foss & Keteyian, 1998). Therefore, subject 01's low iron levels would have affected her aerobic performance (i.e.,

due to decreased oxygen transport and less energy available to do work). As well, research has suggested that many cell-mediated and non-specific immune responses are impaired in iron deficiency (Kuvibidila & Baliga, 2002).

The results from several of the immunological variables also proved to be concerning for subject 01. Specifically, subject 01 experienced a different response to training in terms of white blood cells, S-IgA, and glutamine/glutamate compared to the group as a whole. Firstly, it is important to note that subject 01 had a significantly low white blood cell count during the taper period. The results from the CBC and differential revealed that subject 01 had a white blood cell count of 4.2×10^9/L during the taper, which is below the normal female reference range (Dynacare Kasper Medical Laboratory, 2002). According to Fischbach (1996), a decrease in white blood cells below normal values may occur during viral infections, or with some bacterial infections. This may explain why subject 01 became ill immediately prior to the major competition.

Secondly, the results for S-IgA for subject 01 were very different from those of the group as a whole. Figure 4.71 illustrates clearly how subject 01 experienced much lower S-IgA levels compared to the group. For instance, the S-IgA levels of subject 01 ranged from 43.25 -66.5 ug/ml, while the group mean ranged from 93.8 -158.8 ug/ml. This finding is interesting as several researchers have suggested a link between decreased S-IgA levels and upper respiratory tract infections (Krzywkowski, Petersen, Ostrowski et al, 2001; Gleeson et al, 1995).

Thirdly, the results for glutamine and glutamate for subject 01 were very insightful. Figure 4.73 demonstrates how subject 01's glutamine:glutamate ratio reached its highest level of 14.09 during the build period and then decreased with each consecutive test period,

reaching a low ratio during the taper of 5.1. To date, there has been one previous study that has examined the glutamine:glutamate ratio as a means of tracking training tolerance. This study by Smith & Norris (2000), which examined 52 National team athletes (31 male and 21 female), suggested that very low glutamine:glutamate ratios were indicative of overreaching or overtraining. Unfortunately, the methodology for measuring glutamine and glutamate was different compared to this study; Smith & Norris used a colorimetric assay, whereas high performance liquid chromatography (HPLC) was used in this study. Therefore, a direct comparison of their results with this study's results could not be made.

Another indication that subject 01 had reached a high level of fatigue was the heart rate variability results. Specifically, the results from the dynamic postural heart rate test revealed that subject 01 had a significantly elevated standing heart rate during the taper test. Subject 01 had an average heart rate for the last 30 seconds of the test that was 53.2 bpm during the build period, but then increased to 106.0 bpm during the taper test (see Figure 4.75). As previously discussed in this chapter, an elevated standing heart rate is indicative of sustained sympathetic modulation of heart rate, which is a sign of impending fatigue (Iellamo et al, 2002). However, it should be noted that these results are somewhat inconsistent with the results of the spectral heart rate analysis. During the taper test, the spectral heart rate analysis demonstrated that subject 01 had an increase in her high frequency component of heart rate modulation, which represents an increase in vagal modulation. Nevertheless, the OmegaWave report indicated that subject 01 was slightly tired, and that her cardiac system was "not ready for activities of maximal volume and intensity".

Lastly, subject 01's performance in the test sets, time trials and major competition were the most obvious indications of her level of fatigue. For the 4 x 50m test set, subject 01's performance was essentially the same during the taper period as it was during the baseline period. The 100m time trial also demonstrated similar results (Table 4.49). Most importantly, however, was subject 01's performance at the Canadian National Championships. As was mentioned earlier, she swam considerably slower than usual in the two events that she competed in. Therefore, the 4 x 50m swimming test set, and the 100m time trial appear to be practical means for coaches and athletes to assess levels of fatigue, to monitor training, and to predict competition performance.

CHAPTER SIX: CONCLUSION AND FUTURE DIRECTIONS

OVERVIEW:

This chapter summarizes the findings of this study in terms of the group results, the results for the responders versus non-responders, and also the case study subject. It also provides a general conclusion based on the findings, suggests directions for future research in this area, and provides suggestions for coaches and athletes.

A) SUMMARY OF GROUP RESULTS:

In terms of the group results, analysis of the variables revealed statistically significant differences across testing sessions (based on the results from the repeated measures ANOVAs) for 7 of the variables. Some of these differences may be due to positive training adaptations, while others indicate that the athletes were pushed too far during the body of training or that the taper was not optimal.

There are 3 variables that appeared to show a positive response across the 10-week study. These variables include mean cell volume, the CD4+/CD8+ ratio, and cortisol. Mean cell volume (MCV) significantly increased from the baseline value with training. An increase in MCV was advantageous for the subjects, as research has suggested that younger cells appear to be more efficient in oxygen transport. Further, the group results for the CD4+/CD8+ ratio also suggest that positive adaptations occurred with training. During this study, the group's CD4+/CD8+ ratio was lowest at the baseline testing, and increased with each test, reaching its highest at the crash period. This finding suggests that the athletes experienced enhanced immunocompetence with training. Unfortunately, there were no results for the CD4+/CD8+ ratio during the taper test to confirm this suggestion. Lastly,

the results for the morning cortisol indicate a positive training response to the taper. The concentration of morning cortisol decreased significantly during the taper. According to Bonifazi et al (2000), a decrease in cortisol during a taper is a pre-requisite for improved performance, and could be considered as an index of adaptation to the work load. Unfortunately, these variables are in contrast to several other variables that suggest that the subjects may not have been in an ideal physiological state during the taper period.

Based on analysis of the group results, there are 4 variables (iron, glutamine/glutamate, the predicted 100m and the 100m time trial) that suggest that the training (especially during the overload period) or the taper were not optimal. In terms of the group's iron results, a decrease was observed with training. Although the iron levels were still within the normal reference range (Dynacare Kasper Medical Laboratory, 2002), the decrease would suggest that the iron loss was incompletely matched by iron intake. As well, the results for the group's glutamine/glutamate ratio were concerning. In this study, the subjects' mean glutamine/glutamate ratio decreased significantly, reaching its lowest level during the taper period. According to Smith & Norris (2000), an increase in the ratio should be observed when athletes are in a tapered condition. This is in contrast to the results of this study, which then leads to the conclusion that the athletes in this study were not at an optimal level to perform immediately prior to the major competition. Lastly, the results from two of the performance measures - the predicted 100m and the 100m time trial - suggest that the taper was not optimal for the subjects in general. Specifically, the results of these two tests demonstrate that the athletes swam faster during the crash period compared to the taper period.

The discrepancy in the results for the different variables supports the use of a multidisciplinary approach in order to obtain a clearer picture regarding the effects of training. By examining all of the group's variables together (including the variables that were not statistically significant), it is possible to see patterns that might not otherwise be clearly apparent and to reconcile some of the seeming contradictions. Viewed as a whole, the results of this study suggest that the block of hard training was probably performed too close to the major competition. This speculation is supported by the fact that performance for the majority of subjects was not ideal at the major competition. However, it is important to recognize the limitations of using group results since statistical group averages can mask significant individual variations.

B) ***SUMMARY OF RESULTS FOR RESPONDERS VS. NON-RESPONDERS:***

The examination of the results for the responders versus the non-responders is helpful in terms of delineating normal from abnormal training responses. Upon close analysis of the results for the non-responders, it can be concluded that these 3 athletes were experiencing a very high level of fatigue, especially during the taper period. There are numerous variables that lead to this conclusion. Firstly, the information on the training volume is concerning. Based on the training log books of the subjects, it is clear that the non-responders swam significantly higher volumes than the responders during weeks 3, 5 and the last 4 weeks of the study. The greatest discrepancy was during week 7. As well, the fatigue ratings recorded in the training log books demonstrate that the non-responders had higher fatigue ratings prior to the competition than the responders.

Secondly, the energy intake was considerably lower for the non-responders compared to the responders during the crash period. Specifically, the non-responders consumed approximately 800 less Kcal during the crash period than they did during the build period, whereas the responders consumed relatively the same number of calories at each test period. This information was concerning as inadequate energy intake can be detrimental to athletic performance, and can result in immunosuppression (Venkatraman & Pendergast, 2002; Gleeson & Bishop, 2000).

Thirdly, several of the hematological measures show a difference between the responders and non-responders. The non-responders had lower hemoglobin levels, red blood cells, hematocrit and mean cell hemoglobin concentrations at each of the test sessions. As well, the non-responders had significantly lower iron levels than the responders, especially during the crash and taper periods.

Fourthly, the results for many of the immunological variables show differences between the responders and non-responders across the training season. For example, the responders maintained fairly consistent white blood cell and differential values across the testing sessions, while the non-responders experienced elevated white blood cell and differential values during the crash period, but decreased levels during the taper period. Further, the results for salivary immunoglobin A show that the non-responders had much lower levels during the crash period compared to the responders. This could be problematic as previous research has suggested that a decrease in S-IgA levels may be associated with upper respiratory tract infections (Krzywkowski, Petersen, Ostrowski et al, 2001; Gleeson et al, 1995). Similarly, the results for the glutamine/glutamate ratio for the non-responders are somewhat concerning. During the crash period, the responders experienced

an increase in the ratio, while the non-responders had a decreased ratio. According to Smith & Norris (2000), an increased glutamine/glutamate ratio is seen in athletes who have developed a load tolerance to training. Further, during the taper period, the ratio decreased for the responders and non-responders, but the ratio was lower for the non-responders. This very low level may suggest that the non-responders were in a "grey" area of overreaching. Taken all together, the results for these immunological variables may explain why all 3 of the non-responders became ill either before or immediately after the major competition.

Fifthly, the results for the cardiovascular measures differ between the responders and the non-responders. Specifically, spectral heart rate analysis showed that the responders experienced an increase in their parasympathetic modulation of heart rate during the crash and taper periods. On the other hand, the non-responders had a decrease in parasympathetic modulation during the taper. Research has suggested that training induced bradycardia results from increased cardiac parasympathetic modulation (Uusitalo, Uusitalo et al, 1998). Further, it is interesting to note that the total heart rate variability score for the responders was lowest during the taper period, while it was highest for the non-responders at this time point. According to Norris (2003, personal communication), a lower total heart rate variability score is more desirable for an athlete (i.e. the cardiac system is adapting to the demands of training or at least not being adversely affected).

Lastly, and likely most important, the results from the performance measures show significant differences between the responders and the non-responders. These differences are most apparent in the results from the major competition. At the competition, the 3 responders were able to achieve 100% best times. On the other hand, the non-responders

performed very poorly, and were only able to achieve an average of less than 97% of their best times in their main events. As well, performances at the major competition by both the responders and the non-responders were closely analogous to the times predicted by the 100m time trial and the 4 x 50m test set. For both of these 2 test sets, the non-responders were unable to perform optimally during the taper period. The non-responders achieved times in these tests that were almost the same during the taper as during the baseline testing (e.g. after they had been out of the water for one month).

Therefore, these results taken all together suggest that the non-responders were experiencing a higher level of fatigue compared to the responders, which was ultimately displayed in their performance at the major competition.

C) *SUMMARY OF RESULTS FOR CASE STUDY SUBJECT 01:*

The results for the case study subject (subject 01) also provide support for a multidisciplinary approach. For this athlete, analysis of one variable alone is insufficient to determine overtraining. However, by looking at the results for all of subject 01's variables together, a picture emerges that indicates that the swimmer had reached a very high level of fatigue. Specifically, subject 01 had outlying data for a number of variables including training volume, energy intake, iron, white blood cells, immunolglobin A, glutamine/glutamate, heart rate variability and the swimming test sets. For subject 01, her results were similar to the non-responders as a whole (discussed above), but some of her results were more extreme. Therefore, the case study showed important intra-individual data that was not apparent in the mean of the group, or in the mean of the non-responders. Close examination of subject 01's variables leads to the conclusion that this athlete was probably overtrained.

Despite the limitations of a case study approach, the information gathered for this subject was valuable and will hopefully lead to further research in this area.

D) *CONCLUSION AND DIRECTIONS FOR THE FUTURE:*

In general, this study indicates that there is no single, clear marker of overtraining. Rather, it supports and validates the concept of a multidisciplinary approach as a useful method of monitoring training, and also of determining levels of fatigue. Analyzing a variety of hematological, immunological, hormonal, cardiovascular and performance measures is shown to be beneficial in developing a more complete picture.

Further, this study demonstrates that it is important to examine groups of athletes in various ways: as a group, as sub-groups and also as individuals. For instance, in this study the group results are beneficial to discern the overall trends that took place across different phases of training (i.e. baseline, build, crash and taper). It is important, however, to exercise some caution when interpreting group results because they can mask individual variations. The comparison of the results for the responders versus the non-responders is useful in delineating normal from abnormal training responses. Finally, the case study examination shows how one particular individual responded negatively to training in terms of several of the variables. The results for the case study subject demonstrate the benefit of using a multidisciplinary approach to assist in identifying overtraining.

Lastly, this study identifies certain parameters that show the most promise as potential markers of fatigue and/or overtraining. These variables include salivary immunoglobin A, iron, heart rate variability (as determined through both the dynamic postural heart rate test, and spectral heart rate analysis), the glutamine/ glutamate ratio,

and performance measures. Therefore, future research should include examination of these variables in more subjects, in a variety of sport settings, and for longer periods of time.

E) SUGGESTIONS FOR COACHES AND ATHLETES

In this study, it was somewhat suprising that only 3 of the 14 swimmers were classified as being responders to training. This information indicates that there were issues with the training program and performance levels for the majority of swimmers in this group. This issue needs to be further explored by both the coaches and swimmers, in consultation with sport scientists. It is hoped that the suggestions contained below may help to identify key issues in the training / performance areas for both coaches and athletes.

This study has added to the body of knowledge regarding fatigue and underperformance, and has identified many measures that have the potential to become markers of overtraining with continued research. Unfortunately, many of these measures involve expensive and/or time consuming laboratory techniques (e.g. salivary IgA, glutamine/glutamate, spectral heart rate analysis). These techniques are often not feasible for coaches and athletes, and therefore it is important to determine practical and economical techniques. This study also identified several more pragmatic measures including the training log book, the dynamic postural heart rate test, and the performance measures which appear to be associated with fatigue. Based on the findings of these tests, the following suggestions may be useful for coaches and athletes:

1) coaches should collect baseline measures for later comparisons;

2) coaches should require their athletes to keep daily training log books that record training volume, intensity and fatigue;
3) coaches need to monitor training on a regular basis through performance tests (i.e. time trials, and the 4 x 50m test set);
4) coaches should have their athletes perform the dynamic postural heart rate test regularly to assess cardiovascular fatigue.
5) coaches should be aware of any extraneous factors in their athletes' lives (e.g., diet, sleep, and school) that may add stress and increase the likelihood of overtraining.

If coaches and athletes follow these guidelines, it is very likely that fatigue can be identified before overtraining is reached. Further, if these practical tests indicate a high level of fatigue, then it may be beneficial to employ the more advanced measures including salivary IgA, glutamine/glutamate and spectral heart rate analysis to confirm the beliefs and to potentially diagnose overtraining. The ultimate goal is to minimize the risk of the development of overtraining syndrome through increased education and awareness of the condition by both coaches and athletes, which will hopefully lead to the implementation of appropriate preventative measures in a timely manner tailored to the needs of each individual athlete.

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APPENDIX A: The Major Symptoms of Overtraining

Physiological/Performance

Decreased performance
Inability to meet previously attained performance standards/criteria
Recovery prolonged
Reduced toleration of loading
Decreased muscular strength
Decreased maximum work capacity
Loss of coordination
Decreased efficiency/decreased amplitude of movement
Reappearance of mistakes already corrected
Reduced capacity of differentiation and correcting technical faults
Increased difference between lying and standing heart rate
Abnormal T wave pattern in ECG
Heart discomfort on slight exertion
Changes in blood pressure
Changes in heart rate at rest, exercise and recovery
Increased frequency of respiration
Perfuse respiration
Decreased body fat
Increased oxygen consumption at submaximal workloads
Shift of the lactate curve towards the x axis
Elevated basal metabolic rate
Chronic fatigue
Insomnia with and without night sweats
Feels thirsty
Anorexia nervosa
Loss of appetite
Bulimia
Amenorrhoea/oligomenorrhoea
Headaches
Nausea
Increased aches and pains
Gastrointestinal disturbances
Muscle soreness/tenderness
Tendinostic complaints
Periosteal complaints
Muscle damage
Elevated C-reactive protein
Rhabdomyolysis

Psychological/Information processing
Feelings of depression
General apathy
Decreased self-esteem/worsening feelings of self
Emotional instability
Difficulty in concentrating at work and training
Sensitive to environmental and emotional stress
Fear of competition
Changes in personality
Decreased ability to narrow concentration
Increased internal and external distractibility
Decreased capacity to deal with large amounts of information
Gives up when the going gets tough

Immunological
Increased susceptibility to and severity of illness/colds/allergies
Flu-like illnesses
Unconfirmed glandular fever
Minor scratches heal slowly
Swelling of lymph glands
One-day colds
Decreased functional activity of neutrophils
Decreased total lymphocyte counts
Reduced response to mitogens
Increased blood eosinophil count
Decreased proportion of null (non-T, Non-B lymphocytes)
Bacterial infec5ion
Reactivation of herpes viral infection
Significant variations in CD4:CD8 lymphocytes

Biochemical
Negative nitrogen balance
Hypothalamic dysfunction
Flat glucose tolerance curves
Depressed muscle glycogen concentration
Decreased bone mineral content
Delayed menarche
Decreased haemoglobin
Decreased serum iron
Decreased serum ferritin
Lowered TIBC
Mineral depletion (Zn, Co, Al, Mn, Se, Cu, etc.)

Increased urea concentrations
Elevated cortisol levels
Elevated ketosteroids in urine
Low free testosterone
Increased serum hormone binding globulin
Decreased ratio of free testosterone to cortisol of more than 30%
Increased uric acid production

APPENDIX B: PARTICIPANT INFORMATION SHEET

Faculty of Physical Education and Recreation
University of Alberta
Edmonton, Alberta
T6G 2H9

PARTICIPANT INFORMATION

Fatigue and Under-performance in Elite Competitive Swimmers: Tools for Monitoring Training

Principal Investigator: Karin Van Campenhout, Faculty of Phys. Ed. and Rec. (492-7394).

Supervisors: Dr. Dru Marshall, Faculty of Phys. Ed. and Rec. (492-3615)
Dr. Steve Norris, Faculty of Kinesiology U of C (403-220-7005)
Dr. Vicki Harber, Faculty of Phys. Ed. and Rec. (492-1023)
Dr. Catherine Field, Ag. Foods and Nutrition Sci. (492-2480)

We invite you to take part in a research study at the University of Alberta. Taking part in this study is voluntary. The study is described below. This description tells you about what you will be asked to do, the time involvement required, and any discomfort that you might experience. Feel free to discuss any questions that you might have with the principal investigator.

What is this project about?
Endurance athletes, such as swimmers, try to endure high volumes and intensity of training in order to elicit positive physiological changes. Unfortunately, there is a fine line between positive training, which results in improved performance, and training which leads to performance decrements. Therefore, it is important to develop scientific testing programs that can be incorporated into an athlete's training regime.

Purpose:
This study is being conducted to investigate some of the suggested indicators associated with fatigue and decreased performance including:

- saliva levels of stress hormones (i.e. cortisol)
- blood levels of particular proteins (i.e. glutamine and glutamate)
- various blood markers (complete blood count, iron profile, natural killer cells)
- immune system measures (i.e. salivary Immunoglobin A)
- heart rate variability measures
- mood state, and
- swimming test sets (5 x 200m, 4 x 50m, 100m and 400m time trials)

As well, this study will attempt to identify whether practical and economical tools, such as the swimming test sets and the heart rate variability tests, are effective tools that coaches and athletes can easily use to monitor training.

Who can participate in the study?

You can participate in the study if you are a competitive swimmer between the ages of 16-23 years, currently training with either the University of Alberta Swim Team or the Edmonton Keyano Swim Club (National group). As well, you must qualify to compete at the Canadian National Championships, and have at least 4 years of competitive swimming experience.

What is involved in participation?

To participate in the study, you will undergo 5 testing sessions during a period of approximately 3 months (September 9th, September 12th, early October, early November, and during tapering for Nationals).

You will train according to your own coach, however you will be required to keep a daily training log. In the log book, you will record training volume (distance swum), subjective ratings of training intensity (rated on a 7 point scale for each set), time spent doing dryland activities, feelings of illness and menstrual cycle information (for the females).

Testing will be conducted at the Kinsmen Sports Center and at the University of Alberta. Testing will occur on a Monday between 6 and 8 AM and again between 3 and 4 PM (during your normal training times). On the day before testing (Sunday), you will be asked to provide two saliva samples (one given between 4 and 5 PM and again between 8 and 10PM) which can be done at home. This will require you to spit into a plastic tube, and to store it in your fridge overnight, and then bring it to the pool on Monday. You will also be asked to fast for 8 hours before coming to testing in the morning (which includes no alcohol, caffeine, or cigarettes). When you arrive at the pool in the morning, your height, weight and waist circumference will be taken, and then you will be asked to perform the heart rate variability tests. These tests involve lying on your back for 8 minutes and then shifting to an upright position for 2 minutes. Heart rate response to this posture change will be monitored by electrodes placed around your heart and by a heart rate monitor. Upon completion of this test, you will rest in a chair for approximately 10-15 minutes during which time a recovery-stress questionnaire (RESTQ-76 Sport) will be completed. Following this passive test, a blood sample from your arm vein will be taken by a registered nurse. Once blood has been collected, you will be allowed to warm-up in the pool (1000m in total). After warm-up, you will complete the 5 x 200m test set, followed by a 400m cool down. After this, you will be asked to do a 400m maximal effort time trial. This will conclude the morning testing session. In the afternoon between 3 and 5PM, you will be asked to warm-up for 1000m and then do the 4 x 50m swim test set. This will be followed by 400m of cool-down, and a 100m maximal effort time trial.

Twice during the study, you will also be asked to complete a 3-day dietary record. This requires that you record all the food and beverages that you consume in a logbook for 3 consecutive days. Your records will be analyzed, and you will be provided with feedback regarding your diet.

What is the time commitment?
Most of the testing, other than the first two testing session, will be done during your normal practice times. The 5 testing sessions will take about 3 hours each (2 hours in the morning, and one hour in the afternoon), for a total of 15 hours. You will also need to keep a daily workout log book which should only take you 5 minutes to complete (5 minutes x approximately 84 practices = 7 hours). As well, the dietary records, which are done twice during the study will take you about two hours to complete in total. This gives a grand total of approximately 24 hours.

What if I volunteer and then decide I don't want to continue?
You are required to take part in all of the procedures but you are free to withdraw from the study at any time. You are not required to give a reason, but you must tell one of the researchers. If you withdraw from the study, your information will be removed upon your request.

Are there any risks involved?
The heart rate variability test is a passive, resting test and therefore there are no risks associated with it. The risks pertaining to the RESTQ-Sport questionnaire revolve around the disclosure of personal or sensitive information, which may make you uncomfortable. The swimming time trials (100m are 400m) are maximal effort swims, however the effort required is no greater than what you exert in swimming competitions. The swimming test sets (5 x 200m and 4 x 50m) are submaximal tests, which would involve an effort typical of a regular training session. There are some risks associated with the blood sampling, such as bruising and/or infection at the collection site. Having a nurse trained in taking the blood samples will minimize the risks. As well, a sterile environment and the use of a band-aid will help decrease the risks. Researchers are trained to handle emergencies, and have certification in CPR.

Are there any benefits to participation?
Participating in this study may benefit you during the second half of your competitive season. During the course of the study, your responses to training will be well documented, and the principal researcher will share the results with you and your coach. The information obtained may be beneficial in monitoring your training, and in promoting optimal performance.

How confidential are my results?
Individual data will be coded so that personal identification is not possible. The information will be stored in a locked file cabinet and secure computer to which only the researchers have access. Normally, information will be retained for a period of 5 years post publication, after which it will be destroyed.

What if I have questions or concerns about this research?
If you have any questions about the study and your participation, please feel free to contact one of the researchers. If you wish to speak to someone who is not involved in this study, please contact Dr. Wendy Rodgers, Chair of the Faculty Ethics Committee, Faculty of Physical Education and Recreation at 492-5910.

APPENDIX C: INFORMED CONSENT SHEET

Faculty of Physical Education and Recreation
University of Alberta
Edmonton, Alberta
T6G 2H9

Title of Project:
Fatigue and Under-performance in Elite Competitive Swimmers: Tools for Monitoring Training

Principal Investigator(s): Karin Van Campenhout, Faculty of Phys. Ed. and Rec. (492-7394)

Co-Investigator(s): Dru Marshall, Faculty of Phys. Ed. and Rec. (492-3615)
Steve Norris, Faculty of Kinesiology U of C (403-220-7005)
Vicki Harber, Faculty of Phys. Ed. and Rec. (492- 1023)
Catherine Field, Ag. Foods and Nutrition Sci. (492-2480)

CONSENT FORM:

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached information sheet?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate, or to withdraw from the study at any time, without consequence, and that your information will be withdrawn at your request?	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your information?	Yes	No
Do you understand that you will not be remunerated for participating in this study?	Yes	No

This study was explained to me by: _____

I agree to take part in this study.

_____ _____ _____
Signature of Research Participant Date Witness

_____ _____
Printed Name Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____
Signature of Investigator or Designee Date

APPENDIX D: PARENT OR GUARDIAN CONSENT FORM

Faculty of Physical Education and Recreation
University of Alberta
Edmonton, Alberta
T6G 2H9

Title of Project:
Fatigue and Underperformance in Elite Competitive Swimmers: Tools for Monitoring Training

Principal Investigator(s): Karin Van Campenhout, Faculty of Phys. Ed. and Rec. (492-7394)

Co-Investigator(s): Dru Marshall, Faculty of Phys. Ed. and Rec. (492-3615)
Steve Norris, Faculty of Kinesiology U of C (403-220-7005)
Vicki Harber, Faculty of Phys. Ed. and Rec. (492- 1023)
Catherine Field, Ag. Foods and Nutrition Sci. (492-2480)

PARENT / GUARDIAN INFORMED CONSENT:

Do you understand that your child has been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in your child taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that your child is free to refuse to participate, or to withdraw from the study at any time, without consequence, and that his/her information will be withdrawn at your or his/her request?	Yes	No
Has the issue of confidentiality been explained to you and your child? Do you understand who will have access to your child's information?	Yes	No
Do you understand that your child will not be remunerated for their participation in this study?	Yes	No

This study was explained to me by: _____

I agree to allow my child to take part in this study.

_____ _____ _____
Signature of Parent / Guardian Date Signature of Research Participant

_____ _____
Printed Name Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to the participation of his/her child.

_____ _____
Signature of Investigator or Designee Date

APPENDIX E: SAMPLE DAILY TRAINING LOG BOOK SHEET

Date:_____ Location:_____SC / LC Time:_____AM / PM

Swim Volume:_____(Km)

Swim Intensity: 1 2 3 4 5 6 7

Level of Fatigue: 1 2 3 4 5 6 7

1 = very, very low
2 = very low
3 = low
4 = moderate
5 = high
6 = very high
7 = very, very high

dryland work time: _____

presence of illness: yes no describe if yes:_____

occurrence of menstration: yes no

General Comments?_____
_____

Date:_____ Location:_____SC / LC Time:_____AM / PM

Swim Volume:_____(Km)

Swim Intensity: 1 2 3 4 5 6 7

Level of Fatigue: 1 2 3 4 5 6 7

1 = very, very low
2 = very low
3 = low
4 = moderate
5 = high
6 = very high
7 = very, very high

dryland work time: _____

presence of illness: yes no describe if yes:_____

occurrence of menstration: yes no

General Comments?_____
_____

APPENDIX F: SUBJECT CHARACTERISTICS FORM

SUBJECT CHARACTERISTICS

NAME:_____

DATE:_____

AGE:_____

BIRTHDATE:_____

HEIGHT:_____

WEIGHT:_____

WAIST CIRCUMFERENCE:_____

Medication/vitamins taken in past 3 days:

_____

_____

FOR FEMALES:

Approximate date of last menstrual cycle (first day) : _____

APPENDIX G: STANDARDIZED 1000M WARM-UP

WARM-UP:

- 400m swim
- 200m kick
- 200m drill
- 4 x 50m descend 1 → 4

APPENDIX H: IMMUNOFLUORENSCENCE ASSAY PROTOCOL

Collect blood in 5ml green top tube
Spin blood @ 3000rpm for 10 minutes
Remove plasma and split into 4 or 5 - 500ul and store at -70C for further analysis
Add 2ml 1% BSA in PBS buffer to RBC and buffy coat and gently re-suspend
Place in fridge for storage overnight or start IF

1. Pre-condition plate with 5% FCS in PBS buffer (IF buffer). Fill "V" well plate with 200ul IF buffer and let sit at RT for at least 30 min.
2. Add 100-150 ul of diluted blood to wells (total of 4 well per sample)
3. Lyse cells by adding 150ul lysis buffer to each well and incubate at 37C for 10 min.
4. Spin plate for 2 min (program 9) to pellet cells
5. Suction off lysis buffer, vortex plate
6. Add additional 200ul lysis buffer and incubate an additional 10 min
7. Spin, pellet, suction, and vortex plate
8. If there are still RBC's in the pellet, then repeat
9. Once all the RBC's are gone then wash your plate with IF buffer to remove any lysis buffer (200ul)
10. Spin, pellet, suction, and vortex
11. Add 10ul of AB's (the CD4 and CD16 to well #3 and 4) and add 20ul of buffer to well 1 and 2.
12. Incubate 30min in fridge
13. Wash plate with 200ul IF buffer
14. Spin, pellet, suction and vortex
15. Add 10ul QR strept-avidin to well 2,3,4 (bitoin label)
16. Incubate 30min in fridge
17. Wash plate with 200ul IF buffer
18. Spin, pellet, suction and vortex plate
19. Add 200ul cell fix. Cover in foil and store in fridge.

Well #1 is cells alone (neg control)
Well #2 is Biotin Bkg (neg control)
Well #3 is CD4F and CD8B
Well #4 is CD16F and CD56B

APPENDIX I: INSTRUCTIONS FOR SALIVA COLLECTION

Collection Days :

Please provide two saliva samples on Monday September 9th, and again on Wednesday September 11th.

Time of Collection:

Give the first saliva sample between 4 and 5 PM and then give the second saliva sample between 8 and 10 PM.

Collection Procedure:

1.) Rinse mouth vigorously with water. Wait 5 minutes.
2.) Open stopper, remove cotton swab and place in mouth. Try not to touch the cotton swab if possible.
3.) Chew the swab for 60 seconds.
4.) Return the saturated swab to the vial and close the stopper.
5.) Place the vial into the plastic bag and store it in the freezer.
6.) Bring your vials to the morning testing on Thursday September 12th (trying to keep it frozen).

NOTE: If for some reason you miss a collection, please disregard that particular vial and continue the collection at the next scheduled time.

APPENDIX J: DYNAMIC POSTURAL HEART RATE (RUSKO) TEST RECORDING SHEET

RUSKO HEART RATE TEST

NAME:_____

DATE:_____

TIME	HEART RATE
6 minutes	
6:10	
6:20	
6:30	
6:40	
6:50	
7:00	
7:10	
7:20	
7:30	
7:40	
7:50	
8:00	
8:15	
9:30	
9:40	
9:50	
10:00	

APPENDIX K: Comparison of Predicted 400m Time with 400m Time at Major Competition

Table 1A. Subjects who competed in 400m FR at their major competition compared to their predicted 400m time (from the 5 x 200m test set)

Subject	400m FR time at competition (seconds)	Predicted 400m time (from 5x200)	Time Difference (seconds)
01	290.6	276.0	+14.6
02	263.8	245.2	+18.6
03	236.8	231.6	+5.2
04	247.1	252.8	-5.7
07	237.0	238.0	-1.0
12	246.8	261.6	-14.8
14	277.9	264.8	+13.1
			Average difference: +4.3 seconds

Table 1B. Results from the 5 x 200m test set during the taper period for the 7 subjects who competed in 400m freestyle at the major competition

Subject	slope	r^2	Predicted 100m split at Hr_{max} (sec)	Predicted 400m time (sec)
01	262.2	0.99	69.0	276.0
02	110.4	0.96	61.3	245.2
03	124.8	0.98	57.9	231.6
04	178.1	0.96	63.2	252.8
07	130.3	0.84	59.5	238.0
12	264.5	0.95	65.4	261.6
14	252.4	0.99	66.2	264.8